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THE EFFECT OF HEAT UPON THE MYCELIUM OF CERTAIN STRUCTURAL-TIMBER-DESTROYING FUNGI WITHIN WOOD[1]

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The possible use of heat for checking decay in buildings has been suggested by various European writers, but little has been done either in an experimental or in a practical way to test its efficacy. Falck (1, p. 197; 2, pp. 338–340) made some tests upon the effect of heat upon the mycelium of some wood-destroying fungi in agar and in wood. The tests were not run in a systematic series, and Falck's only conclusion was that, whereas heat may be applicable for the checking of *Merulius lacrymans* within structures, it is not feasible in the case of *Lenzites sepiaria*, for heat is necessary for long periods and at high temperatures to kill this fungus. Hoxie (3, pp. 38–40) reports an experiment carried out in a mill infected with *Merulius lacrymans* and *Coniophora cerebella*, in which encouraging results were obtained by heating the building by means of its own heating system four times from Saturday noon to Monday morning to about 115° F. (46° C.).

The present paper gives the results of a series of tests upon the resistance to heat of the mycelium of certain wood-destroying fungi within wood and a discussion of the application of these results to the heat treatment of decayed timber in buildings, and to the kiln-drying and preservation of wood. The fungi are five found to be of importance in the decay of mill timbers, *viz.*, *Lenzites sepiaria*, *L. trabea*, *Trametes serialis*, *T. carnea*, and *Lentinus lepideus* (6).

METHODS

The cultures of the five fungi were made in 2-liter Erlenmeyer flasks in the manner described by Humphrey (5), using Sitka-spruce blocks $\frac{3}{4}$ by $\frac{3}{4}$ by 2 inches. Tests were made upon blocks from cultures incubated 4 months and from cultures incubated 1 year. In one case the blocks were thoroughly

[1] This work was suggested to the writer by Dr. C. J. Humphrey, begun at the Laboratory of Forest Pathology, Bureau of Plant Industry, Madison, Wis., and finished in the Botanical Laboratory at Brown University.

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invaded by the mycelium and a little decayed, and in the other they were badly decayed and partly dried. Blocks were tested against both dry and moist heat, the moist heat being obtained by supporting the blocks over water in a sealed Mason jar which was placed in the drying oven beside the blocks subjected to dry heat. The blocks to be tested with dry heat were removed from the flasks and air-dried in a warm room for several days, since otherwise, being wet inside, they would for a few hours in reality have been subjected to a moist heat. The blocks to be tested with moist heat were taken fresh from the culture flasks and tested in their partially moist condition. For the tests, the blocks were cut transversely in half, each yielding two pieces $\frac{3}{4}$ by $\frac{3}{4}$ by 1 inch in size. A sufficient number of blocks were placed in the oven at the beginning of the test, and one was removed at the end of each desired interval from 12 hours up to several days, varying with the temperature.

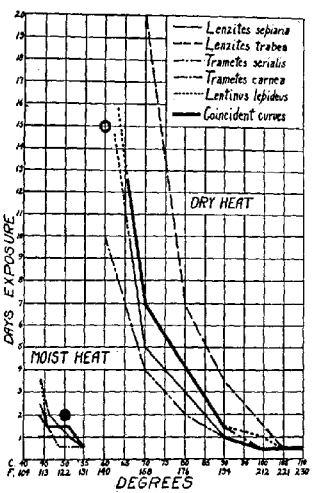
The viability of the mycelium within the block after being subjected to heat was tested by cultures made from the individual blocks. These cultures were made in a culture case kept swabbed with $HgCl_2$, with instruments dipped in alcohol and flamed, upon a block of wood similarly treated. The outer part of the block was flamed, and a slice was removed from each of the six sides. From the remaining inner part, ten pieces of wood material were put in as many tubes of malt agar and set aside to incubate. At least six weeks were allowed for the viable mycelium within the wood to grow out upon the agar.

Results [2]

The results are given in tables 1 and 2 and text figure 1. Inasmuch as little difference was observed between the behavior of mycelium from old and of that from new cultures, the differences are not compiled here. In a few instances, blocks from fresh cultures gave growth where those from old ones did not; in one case the situation was reversed, and in others there was no difference. It was to be expected that some irregularities would occur. For instance, *Lenzites sepiaria* produced no growth at 50° C. moist heat, but one tube of the ten in two tests at 52° C. showed growth. Again, at 52° C. moist heat, old mycelium of *Lentinus lepideus* showed growth after 36 hours' exposure in the first test, but two repetitions with old and one with fresh mycelium failed to show growth. In some tests there might be growth from the block exposed 48 hours and none from the one exposed 36 hours. In the case of the vigorous *Lenzites trabea* at 48° C. moist heat, one test gave no growth at all at 12 hours, while a repetition with blocks from the same culture showed that 24 hours' exposure did not kill the fungus. In the main, however, the results were reasonably uniform.

[2] Some preliminary results were presented in a paper read before the American Wood Preservers' Association (7). At that time it was pointed out that certain of the periods at the lower temperatures, with dry heat (60 percent and 70 percent more particularly), were thought to be incorrect and repetitions were to be made. These questioned tests have been repeated and the discrepancies between the curves in the two papers are thus explained.

In general, the resistance of the mycelium to dry heat was greater than to moist heat, but the degree of difference was somewhat surprising. Three and one half days' exposure to 44° C. and 12 hours at 55° C. moist heat was necessary to kill all the fungi, while with dry heat, the three-day killing period was from above 70° C. to above 90° C. and all the species were not killed in 12 hours until 105° C. was reached. Three of the fungi survived 12 hours at the boiling point of water in dry heat, and one of these (*Lenzites trabea*) survived 24 hours at that temperature.



TEXT FIG. 1. Graph showing the killing point of the mycelium of five "mill-roof" fungi within ¾- by ¾- by 1-inch spruce blocks, by both moist and dry heat. The open circle denotes that three of the fungi—*Lenzites sepiaria, Trametes carnea*, and *Lentinus lepideus*—were not killed at the point marked, which was the last test in that series. The closed circle denotes graphically the maximum amount of heat theoretically available in a mill over the week-end shut-down.

As was to be expected, there was individual variation in the susceptibility to heat of the fungi used. Further, it is apparent that the individual resistances of the mycelia of the different fungi to temperatures above those favorable for growth bear no relation to the temperature curves for growth (*cf.* 6). For example, *Lenzites sepiaria* is a high-temperature organism, having an optimum for growth upon agar between 32° and 35° C., and grows at 40° C., even at 44° C. according to Falck (1, pp. 127–129). The optima for the other fungi are around 28° C. and 30° C. None of the latter will grow at all at 40° C., and some not above 36° C. (6). Yet the mycelium of *L. sepiaria* was killed more easily than that of three of the other fungi and almost as easily as that of the least resistant of the group—*Trametes serialis*.

The mycelium of *Lenzites trabea* was decidedly the most resistant to heat, more especially dry heat. It was killed in 12 hours of exposure to 105° C., but survived one day at 100° C., 3 days at 90° C., 6 days at 80° C., and 19 days at 70° C. The greater resistance of the mycelium of this species was naturally attributed to the possible presence of some resistant spores formed within the wood, but repeated examinations of wood infected with pure cultures of this fungus have not yet revealed the presence of secondary spores, although both oidia and chlamydospores are present in cultures upon agar media. The mycelium of the least resistant of the five (*Trametes serialis*) succumbed after 10 days' exposure at 60° C., 4 at 70° C., 2 at 80° C., and 1 at 90° C. The thermal death points at dry heat for the other three fungi lie nearer to those of *Trametes serialis* than to those of *Lenzites trabea*.

With moist heat, irregularities were more common and several repetitions were necessary to gain an idea of the resistance of the various fungi. Both *Lenzites trabea* and *Lentinus lepideus* in most cases survived 3 days at 44° C., and for varying periods up to 52° C. *Trametes carnea* in part resisted 48° C. for 24 hours, and in one case 52° C. for 12 hours. In these latter tests, however, growth occurred in only 2 or 3 tubes. *Lenzites sepiaria* survived no more than 12 hours at any of the temperatures of moist heat tried, and in one of the two tests at 52° C., growth occurred in only 1 of 10 tubes.

The delaying effect of the more severe conditions, either of length of exposure or of degree of heat, in the growth of the mycelium from the test pieces on to the agar, was quite noticeable in four of the fungi, but less so in *Lenzites trabea*. This species responded to the test-tube conditions almost as well after periods of heat as in control tests. The growth in the tubes was slower in appearing, the more rigorous the exposure. Whereas a good culture may be obtained in 3 or 4 days from unheated wood such as used in these tests, growth from the more severely heated blocks was delayed until 3 weeks or 25 days for four of the fungi. If the mycelium in a block was ever to grow, it always appeared within a month.

Bearing of these Results upon Heat Treatment of Timbers in Buildings

The tests described in this paper were not made with the idea of obtaining practical results regarding the possibility of checking decay in buildings. They were intended simply as preliminary experiments and to be used as a guide in more extended work upon larger pieces of wood. However, even with the small blocks used, the results are so striking as to be worthy of discussion and useful in forming certain conclusions.

These results will apply very well to 1-inch stock, which is usually planed down to $\frac{7}{8}$ inch, because the difference in the time of heating the $\frac{7}{8}$-inch and $\frac{3}{4}$-inch material would be slight. It is quite certain that heat would act more slowly upon mycelium in large beams and planks than it would in the

¾-inch blocks used in the tests. Hence, for example, if different degrees of heat which would be safe to use in buildings would not kill the mycelium in the small blocks, it certainly is hopeless to try heat to sterilize timbers in structures which are infested with the fungi considered here.

In this connection, there are a few things to be considered. One of these is that different wood-destroying fungi react differently to heat. *Merulius lacrymans*, the true dry-rot fungus (or what has thus far been taken for *M. lacrymans*), is a low-temperature organism and is more susceptible to treatment with heat than is the group of organisms considered here. According to Falck (1, p. 129), this fungus (which he calls *M. domesticus*) has an optimum around 21° C. and does not grow at all on agar above 27° C., a point lower than the optimum for any of the five fungi treated in this paper. While it has been pointed out above that there is necessarily no relation between the relative temperature curve and the relative resistance to heat above the growth temperatures (as in the case of *Lenzites sepiaria*), it is known that certain species of Merulius are more susceptible to higher degrees of heat, as would be expected from the growth relations of certain of the species studied. Hence, these tests do not apply to Merulius or to other fungi of the dry-rot group, but apply only to the species considered—those which are adapted to conditions found in cotton-mill weave-shed roofs.

Another consideration is that only dry heat and heat in a saturated atmosphere are considered here. What the effects of heat at the different humidities between the points of dryness and saturation would be can only be conjectured. The same heat applied in a dwelling and in a cotton-mill weave shed would obviously give quite different results because of the difference in relative humidity. The wetness of the wood would also need to be considered. In a dwelling house under ordinary conditions, the wood is below the fiber-saturation point. In a building where the air is saturated, the wood everywhere except in contact with the outside would be only at the fiber-saturation point, while that in contact with the outside would be above fiber saturation when it was cool outside. In one-story weave sheds, the moisture content of the roof planks is much above the fiber-saturation point, because the dew-point often comes within the plank and water is precipitated there. Hence, it is probable that heat applied in a room like a weave shop, even at humidities much below saturation, would act as the moist heat of a saturated atmosphere because of the moisture within the wood.

A further consideration with regard to the practical application of these results to mill-roof conditions is the impossibility of supplying the heat with sufficient uniformity. Although it might be deduced from the tables and graphs that a certain degree of heat for a certain period would kill the mycelium in stock of certain dimensions, it is of course apparent that heat applied in a structure such as a weave shed would be radiated so rapidly

that the available heat for sterilizing purposes throughout a roof plank, for instance, would be much less than that indicated as supplied.

From the practical point of view, the following conclusions may be drawn:

1. Dry heat in buildings in which the air is not artificially humidified is of no use for purposes of sterilizing wood infected with the five fungi discussed here. Too high a degree of heat for too long periods is necessary even in the small blocks to attain the end desired. A heating system in a building is capable of raising the temperature to only about 52° C. (125° F.), and such a temperature would not accomplish the sterilization. If automatic sprinklers are present, a temperature higher than 46° C. (115° F.) would not be safe.

2. Dry heat in dry structures applied for the purpose of drying out the wood and thus preventing or retarding decay is highly to be recommended. Much timber enters a structure in a partially green condition, and such timbers are especially susceptible to decay. A thorough drying before occupation would prevent decay such as often takes place when green or wet lumber is installed. A preliminary drying before painting timbers on the interior of buildings would be good insurance against decay. Green or wet timbers may be expected to decay rapidly in a moist atmosphere unless proper precautions are taken, but they often decay in dry buildings because they are painted over when wet inside. They then rot during the retarded drying and may become a total loss before sufficiently dried. For buildings which are already slowly decaying, a periodic application of dry heat as high and for as long a period as possible would dry the wood and retard the destruction.

3. Dry heat in structures of *moist occupancy* (really moist heat as explained above) would have to be applied as high as obtainable for periods longer than two days to accomplish anything. We speak of two days because that is the time during which heat might be applied in *operating structures*—from Saturday noon until Monday morning. From text figure 1, it is seen that temperatures from 44° to 50° C. (112° to 122° F.) killed the mycelium in the small blocks, but it is certain that not only would it take more heat for larger timbers, but also so much heat would be radiated that more would be necessary than is shown by the graph. Hence, the application of dry heat even in structures where the humidity is high cannot be recommended under ordinary conditions, and it is not certain that long periods of application would be of more value in eliminating these fungi.

4. Moist heat as such (humidified heat or free steam) need not be considered here. In a dry structure, it would be necessary to apply the moist heat for a period sufficient for the moisture to permeate the wood before the killing action took effect, and this would not be practicable under ordinary conditions. Secondly, dry heat applied to wet wood such as is found in weave sheds means that the fungi are in any case being subjected to moist heat within the wood.

Bearing of these Results upon Kiln-drying of Lumber and Structural Timber

The results obtained also throw some light on the effect of kiln-drying of lumber upon the mycelium of fungi within the wood. It is fair to assume that the results with these fungi will apply to the kiln-drying of coniferous timbers. The fungi under discussion in this paper cause most of the serious damage to worked timber, and hence the results obtained are directly

Table 1. *The effect of dry heat upon the mycelium of Lenzites sepiaria, L. trabea, Trametes serialis, T. carnea, and Lentinus lepideus within ¾- by ¾- by 1-inch Sitka-spruce blocks*[3]

		Temperature (degrees C.)						
	Days	60	70	80	90	100	105	110
Lenzites sepiaria	½	+	+	+	+	−		−
	1	+	+	+	−	−		−
	1½	+	+	+	−	−		−
	2	+	+	+	−			
	2½	+	+	+				
	3	+	+	−				
	4	+	+	−				
	5	+	−	−				
	6	+	−					
	7	+	−					
	15	+						
Lenzites trabea	½	+	+	+	+	+	−	−
	1	+	+	+	+	+	−	−
	1½	+	+	+	+	−	−	−
	2	+	+	+	++	−		
	2½	+	+	+	+++	−		
	3	+	+	+	+++			
	3½	+	+	+	−			
	4	+	+	+	−			
	5	+	+	+	−			
	7	+	+	−				
	8	+	+	−				
	9	+	+	−				
	12	+	+					
	19		+					
	20	+	−[4]					
Trametes serialis	½	+	+	+−	+−−	−	−	−
	1	+	+	−−	−	−	−	−
	1½	+	+	+−−	−	−	−	−
	2	+	+	−	−			
	2½	+	+					
	3	+	+					
	4	+	−					
	9	+						
	10	−						
	11	−						
	12	−						

[3] Each plus or minus sign refers to a single test of 10 tubes. A plus sign means that some growth was obtained, whether in one or all ten tubes, but in all but a very few cases it means growth in more than six of the 10 tubes of a single test.

[4] Test not satisfactory. Repetition may show growth.

TABLE 1.—(*Cont.*)

	Days	Temperature (degrees C.) 60	70	80	90	100	105	110
Trametes carnea.....	½	+	+	++	+−−	−	−	−
	1	+	+	++	+−−	−	−	−
	1½	+	+	++	−−	−	−	−
	2	+	+	++−−	−			
	2½	+	+	++−−	−			
	3	+	+	++−−				
	4	+	+	−				
	6	+	+	−				
	7	+	−					
	8	+	−					
	9	+	−					
	15	+						
Lentinus lepideus.....	½	+	+	++	++−	+	−	−
	1	+	+	++	+−−	−	−	−
	1½	+	+	++	−−	−	−	−
	2	+	+	+	−−−	−		
	2½	+	+	+	−			
	3	+	+	+				
	4	+	+	−				
	5	+	+					
	6	+	+					
	7	+	−					
	8	+	−					
	9	+	−					
	15	+						

applicable. If some of the rarer fungi attacking coniferous woods are more resistant to heat than the resistant *Lenzites trabea*, these results and the discussion following do not apply. Just how far they would hold for hardwoods which are infested with different fungi can only be conjectured, although *Lenzites trabea* is common on hardwoods and *L. sepiaria* occasionally occurs on them. In the remainder of the paper, however, it is assumed that by far the greater part of the combat against structural-timber-destroying fungi is against species to which these results are applicable, *i.e.*, the five considered here and others like Merulius which are less resistant to heat. In applying these results to kiln-drying, it is assumed that the effect upon 1-inch stock would be about the same as upon the ¾-inch blocks used in the experiments. In processes in which the kilns are run at a fairly uniform temperature and humidity, the possible killing effect of the heat used may be determined from the curves presented (text fig. 1). In the case of softwoods dried directly from the saw in a green condition with high temperatures, any mycelium should be killed, inasmuch as none of the fungi used, which are the commoner ones, could stand 12 hours at 55° C. moist heat. In the case of hardwoods, the killing of wood-destroying mycelium would depend upon the combination of time and temperature (if not the species of fungus)—especially, perhaps, upon the

TABLE 2. *The effect of moist heat upon the mycelium of Lenzites sepiaria, L. trabea, Trametes serialis, T. carnea, and Lentinus lepideus within ¾- by ¾- by 1-inch Sitka-spruce blocks*

	Hours	Temperature (degrees C.)					
		44	46	48	50	52	55
Lenzites sepiaria	12	+	+	−−	−	++	−
	24	−	−	−	−	−	−
	36	−	−				
	48	−	−				
	60		−				
	72		−				
Lenzites trabea	12	+	+	+−	−+	+−	−
	24	+	++	+−	−	+−	−
	36	+	+	−		−	−
	48	+	−	−		−	−
	60	+	−				
	72	+	−				
Lenzites serialis	12	+−	++−	−−−−	−	−−	−−
	24	+	+++	−−−	−	−	
	36	+	−−	−−			
	48	−	−−				
	60	−					
	72	−					
Trametes carnea	12	+	+	+−	−	+−−	−−
	24	+	+	+−	−	−	
	36	+−	−	−−−			
	48	+	−	−−			
	60	−	−	−			
	72	−	−	−			
Lentinus lepideus	12	+	++	+++	+	+++	−
	24	+	+−	−	−	++−	−
	36	+	−			+−−−	−
	48	+−	−			−−−	−
	60	+	−			−−	−
	72	+−	−			−−	−

time during which the moisture content of the wood was above the fiber-saturation point. It may be seen that it would not take very long periods at temperatures from 45° C. upward, with the wood at fiber saturation (moist heat), to kill all mycelium. Green hardwoods are usually under these conditions long enough to be sterilized within. Preliminary steaming treatments would have the same effect.

In the controlled-humidity type of kiln, the same should hold true. An analysis was made of the curves given by Tiemann (8, Pls. I–VII) which show the conditions operating upon different kinds of 1-inch lumber in a typical run of this type of kiln (table 3). For our purposes here and for comparison with the data presented in this paper, the possibility of killing mycelium in wood in such a kiln may be calculated in two ways: (1) on the basis of dry heat acting over the entire period of the run at the varying temperatures, and (2) on the basis of moist heat acting from the beginning of

TABLE 3. *Data taken from Tiemann's curves of temperature and humidity in characteristic dry-kiln runs (8, pp. 277–285) for comparison with data in text figure 1 to show probable effect of kiln-drying on wood-destroying fungi within the wood*

DRY HEAT [5]

Curve no.	Kind of Wood	Entire Run of Kiln: Temperature (C.)	Entire Run of Kiln: No. of Days	*Probable* Killing Effect as Taken from Curve in Text Figure 1.
1	1-inch hardwood	50–65°	20	None of the fungi would be killed.
2	1-inch hardwood	50–70°	14	None of the fungi would be killed.
3	1-inch hardwood	77–80°	7	None of the fungi would be killed.
4	2½-inch hardwood	43°	64	None of the fungi would be killed.
5	1-inch conifer	75°	10	All but *Lenzites trabea* would probably be killed.
6	1-inch conifer	57–77°	10	No killing effect.
7	1-inch conifer	82–93°	4	*L. trabea* would not be killed, others might be.

MOIST HEAT

Wood at Fiber-saturation Point

1	1-inch hardwood	50°	6	2 days at this temperature would probably kill all fungi.
2	1-inch hardwood	50–55°	8	2 days at this temperature would probably kill all fungi.
3	1-inch hardwood	75° at least	3	12 hours at 55° kills all fungi.
4	2½-inch hardwood	45° at least	25	4 days kills all in ¾-inch material; 25 days should kill all mycelium in 2½-inch stock.
5	1-inch conifer	75°	2–3	2 days at 50° kills all fungi.
6	1-inch conifer	57°	2–5	All killed in 12 hours at 57°.
7	1-inch conifer	82–88°	1½	1½ days at 52° kills all fungi.

the run until the time when the water content of the wood goes below the fiber-saturation point. In the second contingency, the air within the wood is saturated with moisture, and hence the heat applied acts as moist heat regardless of the external humidity, which is, however, high for most of the period.

Taking the results upon five fungi, it appears from the two sets of data (text fig. 1 and table 3) that such a procedure may be counted upon to kill the mycelium of most if not all species in 1-inch stock. Counting the heat applied in these kilns as absolutely dry heat over the whole period (which it certainly is not), it is seen that in most of the cases the mycelium would not be killed. On the other hand, it is seen that moist heat is acting upon the mycelium in the wood in all of these seven conditions (table 3) sufficiently

[5] It is of course understood that the heat for the entire run of the kiln cannot be dry. It is considered as dry for the purposes of this treatment, solely for comparison with the data on dry heat presented and because there are no data available for humidities between dryness and saturation of the air. See text for discussion.

long to kill it. The effect of the same temperatures at intermediate humidities can not be determined.

These results are taken to apply only to 1-inch stock, but with longer periods of treatment of larger material the results should be the same. Moist wood is a fairly good conductor of heat. Furthermore, there is in most cases a sufficient margin between the time necessary to kill the mycelium in 1-inch stock and the time that even the 1-inch lumber is exposed to killing temperatures to allow heat to penetrate to the center of large material and to act upon any mycelium. Most of the decay in structural timbers without doubt owes its incipiency to infection between the time of cutting and the time of installation. These results indicate that kiln-drying could be counted upon to render structural timbers sterile internally as far as most if not all wood-destroying fungous mycelium is concerned, and that in kiln-drying we have an important agent in combating decay in buildings.

Bearing of the Results upon the Possible Sterilizing Effect of Wood-preservation Processes

The results given in this paper indicate also that various wood-preservation processes should be sterilizing processes as well. The necessity for sterilizing treated wood is not everywhere understood. It is often noted that treated wood decays inside in the untreated portion leaving a hollow shell which has been protected. This decay within may have started in two ways. The fungi may have entered through checks or nail holes, etc., *i.e.*, through breaks in the protecting preserved layer, or it may have arisen from fungous mycelium which entered the wood between the time of felling of the tree and the time of preservative treatment. It is certain that a great deal of decay in structural timber owes its origin to this method of infection, as already pointed out. Hence, it is important that the inside of the treated timbers should be sterilized, as otherwise the preservative treatment may be a waste of time and money. This discussion, of course, advocates the treatment of wood already infected only in the sense that all timber apparently sound may be and in a great many cases probably is infected with fungous mycelium before it is installed, and refers only to those preservative treatments in which a fairly high degree of heat is applied. This is very different from even suggesting any kind of preservative treatment, with or without heat, for wood already visibly infected or partially decayed.

Wherever heat is applied, either in a preliminary seasoning treatment or in the preserving process itself, it is possible that the heat will be sufficient to kill any fungi within the wood. It may make some difference whether or not the wood is green or partially dry. If the wood is green, or is wet enough so that the air in the wood cells will be saturated, the heat applied will react as moist heat, and the amount of heat necessary for sterilization purposes under these conditions is not great, since 55° C. for 12 hours will

kill all the fungi tested in $\frac{3}{4}$-inch stock. If the wood is air-dry, it is possible that there will be sufficient moisture in the wood to volatilize under the heat treatment and thus to cause the heat applied to act as moist heat; or the liquid applied may penetrate and produce the same result. Further, of course, it is always possible that the heat may be applied long enough to accomplish the sterilization, even as dry heat.

In such preliminary treatments as applying saturated steam, superheated steam, or hot oil, there should be sufficient heat present to kill all mycelium in wood, especially if aided by subsequent vacuum treatments to rupture the fungous cells. In many preservative processes, the same should be true. For instance, in a treatment such as described by Hoxie (4) for treating material for insulation on a mill roof, the $\frac{7}{8}$-inch pine sap boards were immersed in creosote at a temperature of about 105° C. (220° F.) for 20 hours or more—long enough to accomplish the desired sterilization even on the basis of dry heat, in addition to protecting the wood against future attack. In several pressure processes, the preservative is heated up to 93° C. (200° F.) or above, and the heat is probably high enough for a long enough period to sterilize the wood in many, if not all, cases even on the basis of dry heat.

Summary

Inasmuch as the application of heat to various structures has been suggested as a possible remedy against decay, five fungi found growing in cotton-mill roofs were tested as to their thermal death relations in moist and dry heat. Species of Merulius and other fungi of the dry-rot group are not considered here.

The tests were made upon blocks of Sitka spruce $\frac{3}{4}$ by $\frac{3}{4}$ by 1 inch taken from 4-months- and 1-year-old cultures of the five fungi used and subjected to both moist and dry heat for varying intervals and at varying temperatures.

In moist heat, the most resistant of the fungi was killed in $3\frac{1}{2}$ days at 44° C. and in 12 hours at 55° C. In dry heat, 20 days at 70° C. did not kill the most resistant, nor did 12 hours at 100° C., although all succumbed in 12 hours at 105° C. dry heat.

There were individual differences in the resistance of the various fungi, and the individual curves bore no direct relation to the thermal growth curves. *Lenzites sepiaria* has the highest optimum and maximum of growth of the fungi tested, but next to the lowest thermal death curve. *Lenzites trabea* proves to be by far the most resistant of the five fungi, although its thermal growth relations are about the same as those of the other three fungi.

It is concluded even from the results upon the small blocks that heat applied to buildings as a sterilizing agent can be of little avail against the five fungi tested, although it is pointed out that periodic heatings of such

structures might be of service in checking decay through drying out of the timbers. Heating before structures are painted or occupied is recommended.

Inasmuch as the five fungi tested are the most common destroyers of structural timber and are more resistant to heat than the dry-rot fungi (*Merulius* spp. and others), it is concluded that various kiln-drying and wood-preservation processes should sterilize the wood treated, inasmuch as the data show that sufficient heat is applied in most, if not all, cases to accomplish this result.

DEPARTMENT OF BOTANY,
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AN ISOELECTRIC POINT FOR PLANT TISSUE AND ITS SIGNIFICANCE[1]

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The study of the relation of hydrogen-ion concentration to the growth and development of plants or plant parts has, in many cases, indicated that there is between the extreme limits of acidity or of alkalinity which are injurious to and interfere with growth and development a third region where the reaction affects the plant injuriously. This produces, when the growth of the plant or the intensity of the process is plotted against the hydrogen-ion concentration of the solution, a curve having a double maximum with a minimum located between the two maxima. Webb (25) was apparently the first to call attention to this phenomenon. He found that, when the spores of *Penicillium cyclopium* were germinated in buffer mixtures of H_3PO_4 and NaOH containing mannite, two maxima appeared with a minimum between when the percentage of spore germination was plotted against the pH of the solutions. He found similar curves for *Fusarium* sp., and, under certain temperature conditions, for *Aspergillus niger*. For *Botrytis cinerea* and *Lenzites saepiaria* his curves showed but one maximum. In a more recent paper (26) he has studied the germination of the spores of a large number of fungi in various media the hydrogen-ion concentration of which was adjusted by means of H_3PO_4 and NaOH. In various forms, notably Penicillium and Fusarium, and under certain conditions, two maxima were found in the curve of spore germination plotted against the hydrogen-ion concentration of the medium. In at least one set of conditions, two maxima were found for *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, *Fusarium* sp., *Penicillium italicum*, *Lenzites saepiaria*, and *Puccinia graminis*. Only one maximum was found for *Colletotrichum Gossypii*.

Salter and McIlvaine (22) found a double-maximum curve for the growth of wheat seedlings in solution cultures the reaction of which was adjusted with citric acid and NaOH. They concluded that this curve resulted from the exhaustion of nitrates due to the development of bacteria in the cultures.

[1] Published with the permission of the Director of the Agricultural Experiment Station, University of Missouri. The experiments on the absorption of water by potato-tuber tissue in buffer mixtures of H_3PO_4 and NaOH were performed for the writer by Mrs. Edward Abbott. The assistance of Mr. Karl Petsch in the experimental work involved is also acknowledged.

Hixon (9) investigated the germination and seedling growth of peas, corn, wheat, oats, and carrot in Tollen's solution, in agar made from tap water, in tap water, or in Tollen's solution made up in tap water. In each case the reaction of the medium was adjusted with HCl or NaOH to a pH of approximately 3.0, 4.0, 5.0, 6.0, and 7.0. He found a double-maximum curve for the germination and seedling growth in length of peas with the minimum at pH 5.0. A double-maximum curve was found for the germination and seedling growth in length of corn, wheat, and oats with the minimum at a pH of 6.0 between the two maxima. For the germination of carrots a double-maximum curve with minimum at pH 5.5 was found, although the curve for the seedling growth showed but one maximum. The wet weight and dry weight of the roots of wheat seedlings grown for 16 days showed a minimum at pH 5.25 with increase in weight on either side of the minimum as the alkalinity or acidity increased, the increase being greater on the alkaline side. The ash content was less in the solutions more acid than pH 6.0 and greater in solutions more alkaline than pH 6.0. The growth of the plants tended to shift the reaction of the more acid solutions toward greater alkalinity and that of the more alkaline solutions toward greater acidity. Hixon quotes a suggestion by E. J. Cohn that the minimum point is the isoelectric point of a protein of the cell membrane of the seed.

Olsen (18), according to Arrhenius, found that some plants showed a double-topped curve when the growth was plotted against the pH of the medium. He explained this as due to the presence of two different strains of plants.

Hopkins (11) in this laboratory grew *Gibberella saubinetii* in liquid media the reaction of which was adjusted by means of H_2SO_4 and NaOH or the potassium phosphates, H_3PO_4 and KOH, and on potato-dextrose agar the reaction of which was adjusted by means of lactic acid. He found that the growth showed a double-maximum curve when plotted against the hydrogen-ion concentration of the medium. The minimum for this fungus was found at pH 5.5–6.0. In soil cultures in which the reaction was adjusted by H_2SO_4 and NaOH, or by HCl and NaOH, he also found a double-maximum curve when the rate of germination of wheat or the seedling infection of wheat by *Gibberella saubinetii* was plotted against the hydrogen-ion concentration of the soil. The minimum for the number of wheat seedlings up in 4 days was at pH 5.4–5.6, and for infection at pH 5.2–5.5. The conidial germination of *Gibberella saubinetii* (10) was also found to show a double maximum when the percentage of germination was plotted against the hydrogen-ion concentration of the solution used as a medium for germination.

Cole (5) found that the increase in length of seedling corn roots in a 2–4 day period in buffer mixtures of H_3PO_4 and NaOH, or KH phthalate and NaOH, showed a double-maximum curve when plotted against the pH of the solution. The minimum was located at about pH 6.0.

Arrhenius (1) calls attention to the double-maximum curves secured by Hixon and Salter and McIlvaine and states that the same type of curve is also secured when the growth of older plants is plotted against the pH. He cites data for 2½-months-old plants in soil cultures showing that the relative leaf area of bersim (*Trifolium alexandrium*), barley, corn, and cotton showed curves with double maxima. The minimum between the two maxima for bersim was found at pH 6.0, for barley at pH 8, for corn at pH 5 and pH 7, and for cotton at pH 8. In solution cultures 2½ months old, wheat plants and radish plants showed a double-maximum curve for the weight of roots plotted against the pH, the minimum between the two maxima falling at pH 6.0. The weight of stems of wheat plotted against the pH of the solution also showed a double-maximum curve with a minimum at pH 6.0.

The cause of this double maximum and minimum is suggested by Arrhenius as being due to changed intensity of permeability for the different salt nutrients, or to the fact that the solubility of salts differs at different hydrogen-ion concentrations.

Although attention was directed by Webb, Hixon, Hopkins, and Arrhenius to the breaks or minimum points in the curve of the germination of spores and seeds, of the growth of plants or of infection of host plants by fungi when the intensity of the process is plotted against the pH of the medium in which the plant material is placed, similar observations can be found in the older literature. In these cases the hydrogen-ion concentration was not measured, and the results are therefore less certain in their interpretation.

Fischer (8) found that treatment with dilute acids or with solutions of acid salts markedly increased the germination of seeds of *Sagittaria sagittaria* or *Sagittaria platyphylla*, which showed almost no germination in distilled water or in solutions of neutral salts such as NaCl or $CaCl_2$. Dilutions of KOH or NaOH had the same favorable effect as the acids. Copper sulfate also increased the percentage of germination.

Dachnowski (6) found that in dilute acids, $\frac{N}{800}$, and in dilute alkalies, $\frac{N}{800}$, bean and corn seeds showed a greater maximum water absorption than in distilled water.

F. E. Lloyd (12) studied the growth rates and accompanying phenomena in pollen tubes of *Phaseolus odoratus*. Increasing the concentration of acetic, malic, or citric acid in 40 percent cane sugar increased the rate of growth of the pollen tubes to a maximum at $\frac{N}{3200}$, $\frac{N}{12800}$, or $\frac{N}{12800}$ respectively. Further increase in the concentration of the acid decreased the growth rate. Increasing the amount of NaOH in 20 percent cane sugar also increased the growth rate to a maximum at $\frac{N}{3200}$. Further increase in

the concentration of NaOH inhibited the growth. Although the hydrogen-ion concentration was not measured, it would appear that the growth of pollen tubes of *Phaseolus odoratus* in solutions of varying hydrogen-ion concentration should produce a double-maximum curve if plotted against the pH of the solutions.

Cohen and Clark (4) figure a double-maximum curve for the number of viable cells of *B. dysenteriae* (Shiga) at the end of 5.5 hours in media of different hydrogen-ion concentrations. Hopkins (11) has indicated that the curve given by Brightman, Meecham, and Acree (2) for the growth of *Endothia parasitica* at various hydrogen-ion concentrations may be interpreted as having a double maximum.

Although there is an increasing body of evidence to indicate that the occurrence of a double-maximum curve is to be expected when the influence of hydrogen-ion concentrations upon various physiological functions or processes of plants is investigated, a considerable amount of work has been reported which shows no such phenomenon. This should not be considered as necessarily indicating the non-existence of a double-maximum curve. Many conditions may conceivably conceal or obliterate the minimum located between the two maxima. Thus the pH values of the solutions may be separated so far that the minimum is not found. Growth conditions such as temperature, water supply, container size, or salt content of the medium may so limit the development that the values of the two maxima may lie close to the minimum value and be indistinguishable from it.

A minimum located between two maxima also occurs when the swelling, the osmotic pressure, the viscosity, the electrical conductivity, and the alcohol number of gelatin in solutions of varying reaction is plotted against the reaction of the solution expressed as pH. This minimum is located at the isoelectric point of gelatin, pH 4.7. The possibility that there might be an isoelectric point for living tissue and that the existence of an isoelectric point might account for the double-maximum curves and the minimum to which attention has been called in the discussion given above has led to the investigation which is reported in this paper.

The Isoelectric Point

Before describing the experiments involved in this investigation, a brief statement of the meaning of the isoelectric point will add to the clearness of the later discussion.

The conception of an isoelectric point is due to Hardy, who found that the direction of movement of a protein in an electric field is determined by the reaction of the fluid in which it is suspended. Since a hydrosol in which the particles are electro-positive can be changed by the addition of free acid until the particles become electro-negative, it is evident that there must be some point at which the particles and the fluid in which they are immersed are isoelectric. Michaelis and Mostynski (15) defined the isoelectric point

of a protein as that reaction where the relation of the concentration of the hydrogen ions to the hydroxyl ions in the solution is the same as the relation of the acid-dissociation constant (Ka) of the protein to its basic-dissociation constant (Kb). At the isoelectric point the sum of the number of protein anions is equal to the number of protein cations present, and the sum of the protein ions in relation to the non-ionized protein is at its minimum.

The significance of the isoelectric point for the physical and chemical properties of proteins has been pointed out by Hardy, Procter and Wilson, Loeb (13), and others. At the isoelectric point the physical properties of such a protein as gelatin are at a minimum. Thus, if the swelling of the same quantity of gelatin in solutions of different hydrogen-ion concentrations is measured, it is found that the swelling is least at the isoelectric point, pH 4.7. In solutions of greater acidity or alkalinity than this, the swelling increases to a maximum. The same is true of the viscosity, osmotic pressure, conductivity, and alcohol number of gelatin, as has been pointed out by Loeb. A protein can combine with anions only on the acid side of the isoelectric point and with cations only on the alkaline side of the isoelectric point. In other words, a protein may act as either a base or an acid, the reaction of the solution with the isoelectric point as the critical one determining which it shall be. From the above discussion it is evident that there are available several methods of determining the isoelectric point of a protein. It can be determined by the method of Hardy, *i.e.*, by observing the migration of particles in an electric field in solutions of different pH. It can be determined by measuring the swelling, viscosity, osmotic pressure, or electrical conductivity of the protein. It can also be determined by determining in what H-ion concentrations cations or at what hydrogen-ion concentrations anions do and do not combine with the protein. These methods have largely been developed by Loeb (13) chiefly by a study of gelatin.

Experimental

The experimental work described in this paper includes experiments which deal with the absorption of water by potato-tuber tissue in solutions of different hydrogen-ion concentrations and experiments on the absorption of dyes by plant tissue which had been in contact with solutions of different hydrogen-ion concentration.

Experiments on Water Absorption

Potato-tuber tissue was used for the experiments on water absorption because it appeared to furnish the most easily available supply of fairly uniform material. One experiment was also carried out with beet tissue, but the material showed such great variation, probably due to the fact that stored beets, somewhat wilted, were used, that no conclusions could be drawn from the data and the results are not reported.

In the preparation of the tissue the general methods of Stiles and Jorgensen (23) were followed. By means of a cork borer, cylinders of the potato tissue were cut 1.5 cm. in diameter. These cylinders were then sliced into circles about 1 mm. in thickness. At first the slices were cut by hand with a razor. Later a slaw-cutter was used with part of the board cut away under the knife to permit the slices to cut cleanly. The slaw cutter produced pieces of uniform thickness and was much more rapid than the razor. After being cut, the potato slices were rapidly washed with distilled water to remove the starch set free in the cut cells and then blotted dry with filter paper until no evidence of free water was visible on the surface of the slices. After thorough mixing, they were weighed out in lots of 10 g. and dropped into 150-cc. quantities of the solutions used, contained in 150-cc. beakers of Pyrex glass.

Buffer mixtures of phosphoric acid and sodium hydroxide, of secondary sodium citrate and sodium hydroxide, or of potassium hydrogen phthalate and sodium hydroxide were used to maintain the desired hydrogen-ion concentrations. The disadvantages and advantages of using buffer mixtures instead of acids or alkalies only for studying the effect of hydrogen-ion concentration are well appreciated and need not be discussed at length here. The presence of ions other than the hydrogen and hydroxyl ions at concentrations which are necessarily not constant and which are far above those of the hydrogen and hydroxyl ions is unavoidable, and must be taken into consideration in interpreting the data. The buffer mixtures afford a means, however, of maintaining a definite hydrogen-ion concentration which could not be done if an acid or alkali alone were used.

After the potato tissue had stood in the buffer mixtures for 6 to 12 hours, the slices were removed, carefully blotted dry with filter paper, weighed, and returned to a fresh quantity of the same buffer mixture. After 12 or 24 hours, and again at the end of 24 or 48 hours, the process was repeated. In this way three sets of weighings at different intervals of time were made on each lot of potato.

Each treatment was performed in triplicate, and the average of the triplicate weighings is given in the tables or used in constructing the curves which follow. The hydrogen-ion concentration of the solutions in contact with the potato was also determined by Gillespie's colorimetric method each time the potato was weighed in order to learn how much the presence of the potato had affected the reaction. The accuracy with which the colorimetric method of determining the hydrogen-ion concentration was used probably did not exceed 0.1 pH. Difficulty was experienced with brom-cresol-purple. This indicator seemed to yield results which were about 0.2 pH lower than those secured with methyl red or brom-thymol-blue. Wherever possible, determinations were checked with a second overlapping indicator, but in the lower range of brom-cresol-purple this was impossible. All experiments were carried out at room temperature. This varied from

24 to 28° C., but was fairly constant for a given set and was of course uniform in its effect since one set was completed at the same time.

The amount of water absorbed or lost by the potato tissue was assumed to equal the difference between the original weight and that found at the given interval. This is not strictly correct. The absorption of salts from the solution or the loss of salts or organic matter from the potato will affect the weight of the potato. It is believed, however, that the amount of such exchange plays but a small part in the changes in weight which the potato tissue shows in these experiments. Ash determinations made in some of the experiments indicate that fact.

A second source of error, and one which can not be overcome, is the relation of the hydrogen-ion concentration of the interior of the potato cells to that of the solution by which they are bathed. Are they the same or different, and how long does it take for equilibrium to be reached? It is very probable that the H-ion concentration of the interior of the cells is not the same as that of the solution in which they are immersed. Because of the Donnan equilibrium, as has been pointed out by Loeb (13), the H-ion concentration of gelatin chloride in a collodion sac immersed in HCl is less than that of the HCl which surrounds the sac. We should, therefore, expect that the pH of the interior of the potato cells would not be the same as that of the outer solution when equilibrium is reached. No attempt has been made to discover the rate at which the H and OH ions penetrate the potato cells. However, it is probable that they enter rapidly if we can judge from the rapidity with which the color of the cell sap changes when pigmented cells are mounted in dilute acid or alkali. The use of discs about a millimeter in thickness avoided a mass of tissue in the center which would be slowly affected and assisted rapid equilibrium.

Phosphoric Acid—Sodium Hydroxide Series

The buffer mixtures in this series were prepared by mixing 0.1 M H_3PO_4 and 0.1 M NaOH in the proportions indicated by the titration curve for phosphoric acid and potassium hydroxide given by Clark (3). One experiment was performed using the concentrations secured by the use of the 0.1 M H_3PO_4 and NaOH. A second experiment was performed in which the original mixtures were diluted ten times with distilled water, and a third experiment was completed in which the diluted buffer mixtures were used but the range of hydrogen-ion concentration covered included only the region of pH 5.4–6.6. In the first two experiments the distilled water used contained calcium salts carried over in distillation sufficient to raise the pH to 9.0. In the third experiment water redistilled from acid and alkaline potassium permanganate was used in preparing the solutions. The calculated concentrations of H_3PO_4 and NaOH in mols per liter present in each of the buffer mixtures used in the first experiment are given in table 1. The concentrations given there represent those in the final mixtures. Thus,

in solution 3, equal parts of 0.1 M H_3PO_4 and 0.1 M NaOH were mixed. The final buffer mixture contained, therefore, if the two constituents which compose it are considered individually, 0.05 M H_3PO_4 and 0.05 M NaOH. It is recognized, of course, that the H_3PO_4 and NaOH do not exist as such in the buffer mixtures, but for convenience they are expressed as individuals. In the second experiment the concentrations were one tenth of those in experiment one.

In the first experiment of this series the potato was weighed at the end of 8, 12, and 24 hours. The amount of potato used for the solutions of different hydrogen-ion concentration varied from 10.06 g. to 10.16 g., but the gains or losses in weight were calculated per 10 g. of original potato tissue. An examination of the data showed that little change took place in the pH of the solutions as a result of standing in contact with the potato. There was a slight change toward greater alkalinity at the acid end. The increase in weight for the potato in the two most acid and the two most alkaline solutions was greatest at the end of 8 hours. For the balance of the solutions little change took place between 8 and 12 hours. By the end of 24 hours the potato in all solutions had begun to decrease in weight below the maximum. This loss was greatest in solutions 1, pH 2.0, and 3, pH 4.1, amounting to more than 0.9 g., and least in solution 5, pH 5.4, where it amounted to but

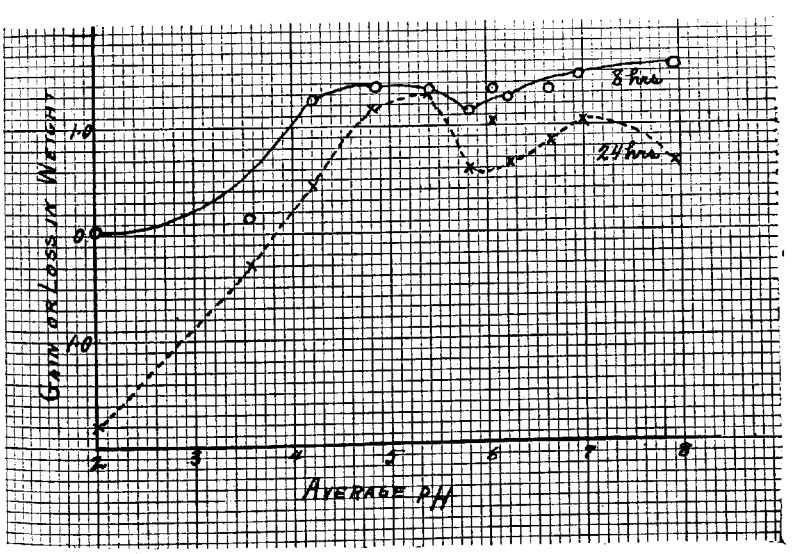

FIG. 1. The gain or loss in weight of 10 g. of potato-tuber tissue in 8 and 24 hours in 150-cc. quantities of buffer mixtures of 0.1 M phosphoric acid and 0.1 M sodium hydroxide. The buffer mixtures changed at 8, 12, and 24 hours.

0.09 g. It is noteworthy that in this solution the weight of the potato remained almost constant at all three weighings. In the other buffer mixtures the loss in weight varied from 0.29 g. to 0.66 g. with the majority around 0.50 g.

If the gain or loss in weight is plotted against the average hydrogen-ion concentration of the solutions expressed as pH, the curves given in figure 1 are obtained. The results secured at the end of 8 and 24 hours are given there. The curve for the 12-hour period is very similar to that for 8 hours, and to avoid confusion is not included in the figure. An examination of figure 1 shows that a smooth curve drawn through the points forms a curve in each case with a double maximum, a minimum occurring between at a pH of 5.8 to 6. If all points were connected, a W-shaped curve would result around pH 6.0 with a peak at pH 6.05. This has not been done in the figure. A consideration of the probable error showed that the minimum at pH 5.8–6.0 could not be accounted for by variation.

In order to reduce the effect of the ions other than H and OH present in the buffer mixtures, the solutions described above were diluted 10 times for the second experiment. The results are given in table 2. There it can be noted that, with less buffer action due to the dilution, the potato produced a greater change in the reaction toward alkalinity at the acid end of the series than was noted with the more concentrated mixture. A change in

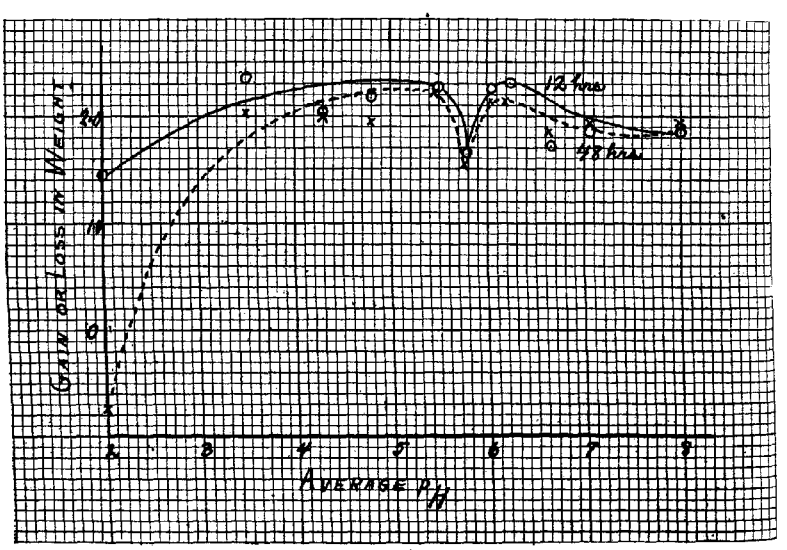

FIG. 2. Gain or loss in weight of 10 g. of potato-tuber tissue in 12 and 48 hours in 150-cc. quantities of buffer mixtures of 0.01 M phosphoric acid and 0.01 M sodium hydroxide. The buffer mixtures replaced at 12, 24, and 48 hours. See table 2.

reaction is not noted to the same extent or regularity in buffer mixtures of pH 5.8 and above. The increase in weight in this series was decidedly greater than with the stronger salts used in the first experiment. There was also a small continued increase at the end of 24 hours instead of the loss noted before. At the end of 48 hours, however, the potato in all solutions showed a decrease in weight below the maximum attained. This loss was again greatest at the acid end and least in the solution of pH 5.4, being but 0.03 g. there, while it was from 0.11 to 0.68 g. in the other buffer mixtures. The potato tissue in this solution again remained almost constant in its gain at the 12-, 24-, and 48-hour weighings.

If we plot the gain or loss in weight against the average pH of the solutions and draw a smooth curve through the points secured, as has been

TABLE 1. *The molecular concentration of the two constituents of the buffer mixtures used in experiments on water absorption*

Sol. No.	Phthalate		Citrate I		Citrate II		Phosphoric Acid	
	KH Phthalate	NaOH	Na Citrate	NaOH	Na Citrate	NaOH	H_3PO_4	NaOH
	mol.	mol.	mol.	mol.	mol.	mol.	mol.	mol.
1	0.01	0.00008	0.00980	0.00020	0.00200	0.00000	0.0714	0.0285
2	0.01	0.00150	0.00950	0.00050	0.00196	0.00004	0.0510	0.0488
3	0.01	0.00354	0.00900	0.00100	0.00180	0.00020	0.0500	0.0500
4	0.01	0.00599	0.00800	0.00200	0.00160	0.00040	0.0495	0.0504
5	0.01	0.00797	0.00700	0.00300	0.00140	0.00060	0.0490	0.0509
6	0.01	0.00860	0.00600	0.00400	0.00120	0.00080	0.0481	0.0519
7	0.01	0.00870	0.00575	0.00425	0.00115	0.00085	0.0471	0.0528
8	0.01	0.00890	0.00550	0.00450	0.00110	0.00090	0.0454	0.0545
9	0.01	0.00920	0.00525	0.00475	0.00105	0.00095	0.0417	0.0583
10	0.01	0.00940	0.00510	0.00490	0.00102	0.00098	0.0384	0.0615
11							0.0333	0.0666

TABLE 2. *The gain in weight of 10 g. of potato-tuber tissue in 12, 24, and 48 hours in 150-cc. quantities of buffer mixtures of 0.01 M H_3PO_4 and 0.01 M NaOH, the buffer mixtures being replaced with a fresh supply at each weighing*

Sol. No.	Initial pH	pH at End of 12 Hrs.	pH at End of 24 Hrs.	pH at End of 48 Hrs.	Gain or Loss, 12 Hrs.	Gain or Loss, 24 Hrs.	Gain or Loss, 48 Hrs.
					g.	g.	g.
1	2.0	2.0	2.0	2.0	1.41±.036	0.14±.02	−0.72±.06
2	3.5	3.5	3.5	3.5	2.33±.046	2.39±.06	2.05±.02
3	4.1	4.5	4.5	4.3	2.02±.08	2.36±.09	2.01±.02
4	4.7	4.9	4.8	4.9	2.18±.036	2.21±.01	1.94±.04
5	5.4	5.6	5.5	5.6	2.25±.01	2.31±.03	2.29±.01
6	5.8	5.7	5.75	5.8	1.66±.07	1.88±.05	1.51±.009
7	6.0	6.1	6.1	6.1	2.25±.04	2.38±.01	2.10±.04
8	6.2	6.2	6.4	6.2	2.30±.02	2.31±.04	2.16±.04
9	6.6	6.7	6.9	6.8	1.70±.05	1.99±.13	1.88±.04
10	7.0	7.1	7.1	7.1	1.83±.06	2.06±.12	1.91±.03
11	8.0	8.0	8.0	8.0	1.82±.03	2.09±.08	1.94±.04
12	9.0	9.0	9.0	9.0	2.23±.02	2.66±.05	1.47±.006
Dist. H_2O							

done in figure 2, we secure a curve having a double maximum with a minimum between at a pH of 5.75–5.8. The curves given in figure 2 are those for 12 and 48 hours.

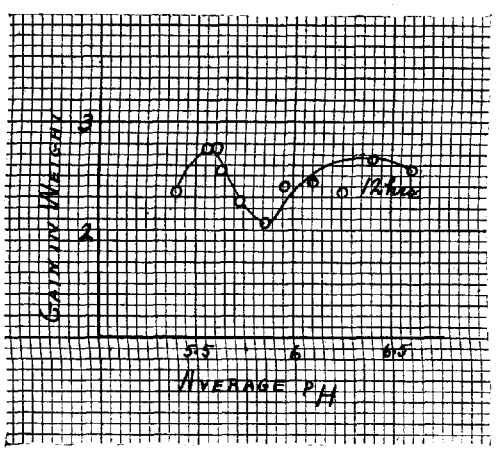

FIG. 3. The gain in weight of 10 g. of potato-tuber tissue in 150-cc. quantities of 0.01 M phosphoric acid and 0.01 M sodium hydroxide in 12 hours.

In the third experiment the dilute buffer mixtures were again used, but what appeared to be the critical region of hydrogen-ion concentration was covered with a greater number of solutions. In this case the reaction at the alkaline end of the series changed toward greater acidity. This change was evident in solutions of pH greater than 5.75. An examination of the in-

TABLE 3. *Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M Na citrate and 0.01 M NaOH, the buffer mixtures being replaced with fresh quantities at each weighing*

Sol. No.	Initial pH	pH after 6 Hrs.	pH after 12 Hrs.	pH after 24 Hrs.	Gain or Loss, 6 Hrs.	Gain or Loss, 12 Hrs.	Gain or Loss, 24 Hrs.
					g.	g.	g.
1	5.1	5.4	5.4	5.3	0.158	−1.192	−1.925
2	5.2	5.4	5.4	5.4	0.248	−1.410	−2.068
3	5.5	5.5	5.5	5.5	0.066	−1.608	−2.067
4	5.67	5.67	5.67	5.67	0.125	−1.525	−2.083
5	5.8	5.8	5.8	5.8	0.316	−1.258	−1.947
6	6.0	6.0	6.0	5.8	0.333	−1.242	−2.092
7	6.1	6.2	6.0	5.8	0.558	−1.017	−1.767
8	6.2	6.3	6.0	5.8	0.566	−0.825	−1.758
9	6.4	6.3	6.2	6.0	0.716	−0.750	−1.617
10	6.7	6.8	6.6	5.8	1.000	−0.125	−1.250
11	6.8	6.6	6.7	6.4	1.566	1.442	1.583
Redist. H_2O							

creases in weight showed that they were comparable in amount to those secured in the former experiment with the more dilute buffer mixtures of H_3PO_4 and NaOH. In the first four solutions, pH 5.4–5.6, the potato continued to gain in weight for the 48 hours. Solutions 5–10, pH 5.75–6.6, showed losses in weight between the 24- and 48-hour weighings. Again it was noted that in the solution of original pH 5.4 the change in weight was

TABLE 4. *Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M KH phthalate and 0.01 M NaOH*

Sol. No.	Initial pH	pH after 6 Hrs.	pH after 12 Hrs.	pH after 24 Hrs.	Gain or Loss, 6 Hrs.	Gain or Loss, 12 Hrs.	Gain or Loss, 24 Hrs.	Ash, 24 Hrs.
					g.	g.	g.	g.
1	4.2	4.6	4.6	4.6	1.092	−0.900	−2.183	0.0111
2	4.6	4.8	4.7	4.7	1.242	−0.150	−1.933	0.0149
3	4.9	5.2	5.2	5.0	1.367	0.575	−1.408	0.0217
4	5.5	5.6	5.5	5.6	1.400	0.570	−0.983	0.0241
5	5.85	5.85	5.8	6.0	1.392	0.942	−0.008	0.0349
6	6.2	6.2	6.2	6.2	1.333	0.800	−0.492	0.0394
7	6.2+	6.2+	6.3	6.2	1.442	1.025	−0.267	0.0358
8	6.3	6.3	6.4	6.2	1.492	1.142	−0.050	0.0314
9	6.9	6.4	6.4	6.2	1.433	1.033	−0.108	0.0359
10	7.6	6.5	6.6	6.2	1.408	0.925	−0.150	0.0383
11	6.6	6.2	6.3	5.8	2.097	2.008	1.550	0.0409
Redist. H_2O								

least between the 24- and 48-hour weighings. In figure 3 the gains in weight at the end of 12 hours are plotted against the average pH of the solutions. If a smooth curve is drawn through these points, we have again a double maximum with a minimum between at a pH of 5.85. If all points were connected, a W-shaped curve with the peak at 6.05 would be obtained.

Citrate—NaOH Series

The buffer mixtures used in this series were prepared by mixing 0.1 M secondary sodium citrate and 0.1 M NaOH as proposed by Sorensen following the tables given by Clark (3), and diluting the solutions so secured either 10 or 50 times. Redistilled water was used in preparing these buffer mixtures. The calculated concentrations of the secondary sodium citrate and sodium hydroxide used in these two dilute buffer mixtures are given in table 1.

In the first experiment the original buffer mixtures diluted 10 times were used. This was equivalent to mixing 0.01 M secondary sodium citrate and 0.01 M NaOH. The potato was weighed at the end of 6, 12, and 24 hours. At the first weighing the solutions from 4 to 9 were slightly turbid due to the development of bacteria, while the rest were clear. The potato appeared to be in good condition. The bacterial development was about the same at the second weighing, but the potato was quite flaccid especially at the

acid end. At the 24-hour weighing the development of bacteria was marked and the potato was very flaccid in all cases except in the redistilled water. The results of this experiment are given in table 3. In this case the presence of the potato shifted the pH toward greater alkalinity in the first two solutions, pH 5.1–5.2, and toward greater acidity in the last six, pH 6.0–6.8. In solutions 3, 4, and 5, pH 5.5–5.8, no change was noted.

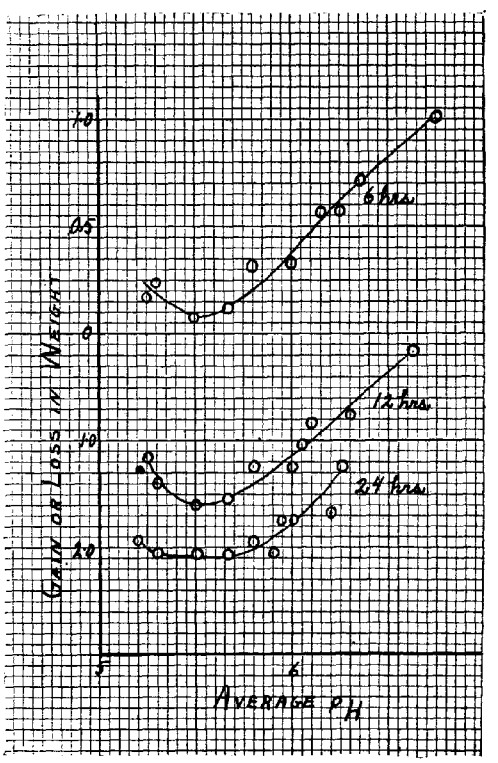

FIG. 4. Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M secondary sodium citrate and 0.01 M sodium hydroxide. Buffer mixtures replaced at 6, 12, and 24 hours. See table 3.

When the changes in weight were examined it was found that the increase in weight was considerably less than with the phosphate series of buffer mixtures. The potato in all the buffer mixtures showed losses by the end of 12 hours which increased in amount by the end of 24 hours. This was evidently due to the citrate ion, as the tendency for the increase in weight was to become greater or the loss in weight less as we proceed toward

the alkaline end of the series where the concentration of the citrate present was less. The loss between the 12- and 24-hour periods was least in solution 3, pH 5.5, where it was 0.46 g. as compared to losses of 0.658–1.125 g. in the other buffer mixtures. If we plot the gains or losses in weight against the average pH of the solutions, as in figure 4, we obtain a curve with a minimum at pH 5.5–5.67. This series would also probably have given us a double-maximum curve had we been able to continue it to greater extremes of acidity and alkalinity.

TABLE 5. *The amount of change in weight expressed as percentage of the total weight and as percentage of gain in weight found between the minimum and the acid or alkaline maximum in the experiments on water-absorption by potato-tuber tissue*

	pH of Acid or Alkaline Maximum	pH of Minimum	Time	Amt. of Change in Weight in Reaching Minimum	
				% Maximum Total Weight	% Maximum Gain
0.1 M H_3PO_4	4.85	5.75	8 hrs.	2.1	17
0.1 M NaOH	7.95	5.75	8 hrs.	3.8	28
0.01 M H_3PO_4	5.5	5.75	12 hrs.	4.9	26
	3.5	5.75	12 hrs.	5.5	29
0.01 M NaOH	6.2	5.75	12 hrs.	5.2	27
0.01 M H_3PO_4	5.6	5.85	12 hrs.	5.7	26
0.01 M NaOH	6.4	5.85	12 hrs.	5.1	24
0.01 M secondary Na citrate	5.3	5.5	6 hrs.	0.9	73
0.01 M NaOH	6.75	5.5	6 hrs.	8.5	93
0.002 M secondary Na citrate	5.6	5.8	6 hrs.	1.6	11
0.002 M NaOH	6.35	5.8	6 hrs.	2.1	10
0.01 M KH phthalate	5.55	6.2	6 hrs.	0.6	4
0.01 M NaOH	6.3	6.2	6 hrs.	1.4	11

A still more dilute series of citrate buffer mixtures was used because of the rapid incidence of loss in weight which took place in the stronger series used first. The second series comprised the original mixtures diluted 50 times instead of 10 times. This was equivalent to mixing 0.002 M secondary sodium citrate and 0.002 M NaOH. The potato tissue was weighed at the end of 6, 12, and 24 hours. The solutions at the end of 6 hours were clear, and the potato was in good condition. There was slight evidence of bacterial action at the second weighing, and, while most of the discs were in good condition, some flaccid ones were present in each lot. By the end of 24 hours all the solutions were turbid, indicating the growth of bacteria, and the potato was flaccid. In this case solutions 1, 2, 3, 4, and 5, pH 5.4–5.8, became more alkaline as a result of standing in contact with the potato, the change being greater in the more acid solutions. In solutions 6, 7, 8, 9, 10, pH 6.1–6.8, the shift was toward greater acidity, the greater change taking place in the solutions of higher alkalinity.

An examination of the changes in weight showed that much greater increases took place with this more dilute solution than were found with the stronger one. However, the maximum was past before the 12-hour weighing, and by 24 hours the potato in all the buffer mixtures save the most alkaline showed a loss in weight. This loss between the 12- and 24-

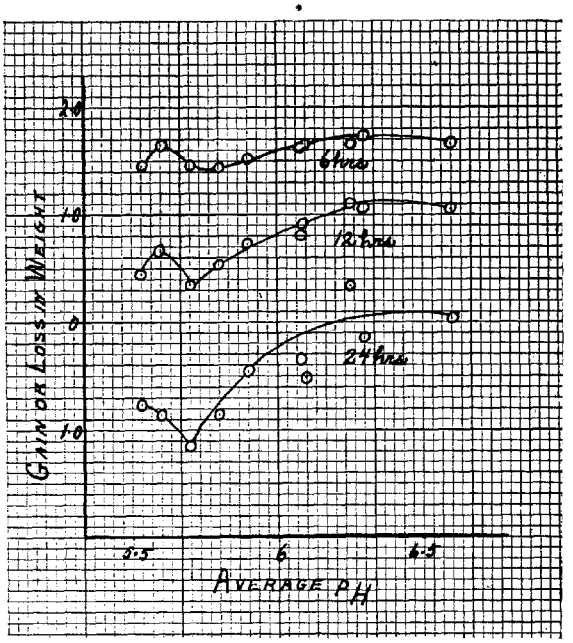

FIG. 5. Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.002 M secondary sodium citrate and 0.002 M sodium hydroxide. Buffer mixtures changed at 6, 12 and 24 hours.

hour weighings was least in solutions 10, 6, and 5, pH 6.8, 6.1, and 5.8. Examining the curve of gain or loss in weight plotted against the pH (fig. 5), a minimum was found at 5.7–5.8, at the 6-, 12-, and 24-hour weighings. The curves at all three periods are much alike.

Phthalate—NaOH Series

The solutions obtained by mixing 0.1 M KH phthalate and 0.1 M NaOH as proposed by Sorensen, using the tables given by Clark (3), were diluted 10 times. The dilution raised the pH of the more acid solutions and lowered that of the more alkaline. The potato was weighed at the end of 6, 12, and 24 hours, and the data are presented in table 4. At the end of 6 hours,

when the first change in weight was measured, the solutions were clear and the potato was in good condition. At the time of the second weighing the solutions were clear, but the potato was flaccid in the acid solutions but turgid and in good condition in the alkaline solutions. At the end of 24 hours, solutions 3–11 were quite cloudy, indicating the growth of bacteria, and all the potato was flaccid save that in redistilled water. It can be noted in table 4 that solutions of pH 4.2–5.85 became more alkaline in contact with the potato, the greater change being at the acid end. Solutions of pH 6.2 and 6.3 showed no change, while the two most alkaline, pH 6.9 and 7.6, showed a decided change toward greater acidity. The changes in weight were quantitatively much the same as those found with the more dilute citrate buffer mixtures. By the end of 24 hours, the potato in all the buffer mixtures weighed less than it did originally. When the change in weight is plotted against the pH of the solution, a curve (figure 6)

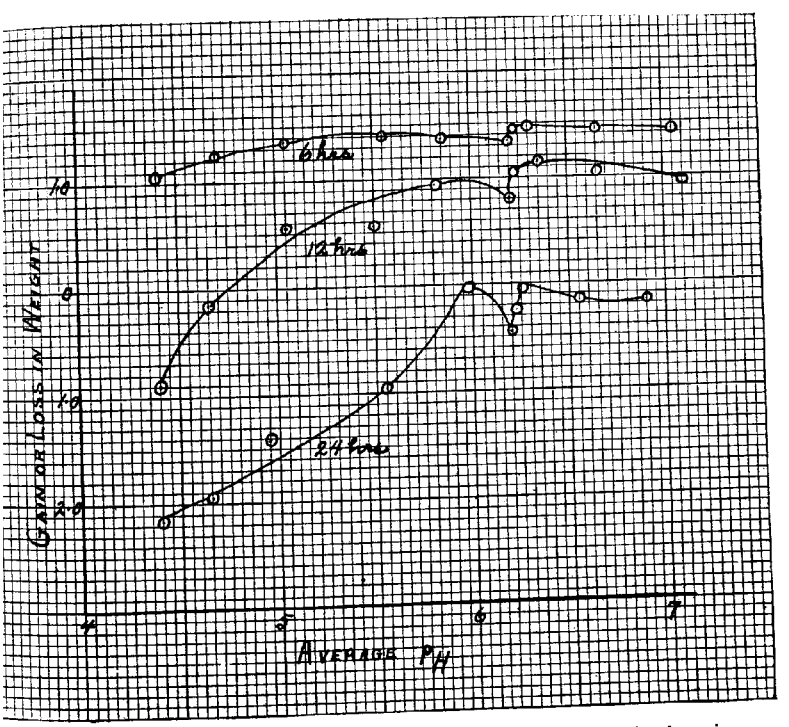

FIG. 6. Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M potassium hydrogen phthalate and 0.01 M sodium hydroxide. See table 4.

TABLE 6. *The regions in each experiment on water-absorption where no change in reaction took place, or where a change toward greater alkalinity or acidity occurred in the buffer mixtures, in consequence of the presence of the potato tissue*

Buffer Mixtures	Reaction Changed toward Greater Alkalinity in Solutions of pH	No Change in Reaction in Solutions of pH	Reaction Changed toward Greater Acidity in Solutions of pH
0.1 M H_3PO_4–0.1 M NaOH	3.5–5.4	5.8–7.9	
0.01 M H_3PO_4–0.01 M NaOH	4.1–5.4	5.8–8.0	
0.01 M H_3PO_4–0.01 M NaOH	5.4	5.6	5.75–6.6
0.01 M Na citrate–0.01 M NaOH	5.1–5.2	5.5–5.8	6.0–6.7
0.002 M Na citrate–0.002 M NaOH	5.4–5.8	6.1	6.3–6.8
0.01 M KH phthalate–0.01 M NaOH	4.2–5.5	5.85–6.3	6.9–7.6

TABLE 7. *The original pH values of the buffer mixtures used in the experiments with dyes, together with the pH of the solutions after standing in contact with the potato and the average; the pH was estimated where starred*

	Buffer Mixtures of 0.02 M H_3PO_4–0.02 M NaOH			Buffer Mixtures of 0.002 M H_3PO_4–0.002 M NaOH.					Buffer Mixtures of 0.002 M Na Citrate–0.002 M NaOH		
Sol. No.	Original pH	Final pH	Ave. pH	Original pH	Final pH, 3 Hrs.	Ave. pH for 3 Hrs.	Final pH, 5 Hrs.	Ave. pH, 5 Hrs.	Original pH	Final pH, 6 Hrs.	Ave. pH
Redist. H_2O											
1	6.5	6.3	6.4	6.6	6.4	6.5	6.4	6.5	6.4	6.3	6.35
2	2.0*	2.0*	2.0*	2.0*	3.7	2.85	4.1	3.05	5.4–	5.4	5.4
3	3.1	3.5	3.3	4.2	5.6	4.9	5.7	4.95	5.4	5.5	5.45
4	4.6	5.1	4.85	5.6	5.8	5.7	5.9	5.75	5.67	5.67	5.67
5	5.6–	5.6	5.6	5.8	5.9	5.85	5.8	5.8	5.8	5.8	5.8
6	5.8+	5.8+	5.8+	6.1	6.1	6.1	5.8	5.9	6.2	6.2	6.2
7	7.0	6.9	6.95	7.0	6.8	6.95	6.4	6.7	6.3	6.2	6.25
8	8.0	7.6–	7.8	7.7	7.6	7.65	6.8	7.25	6.5–	6.5	6.5–
9	9.0	8.05	8.52	7.9–	7.6	7.75	6.9	7.4	6.6	6.4	6.6
10	above 9.75+	8.15	above 8.95	8.1	7.6	7.85	6.8	7.45	6.7	6.6	6.65

is obtained with a minimum at pH 6.2–6.25. The curves for the three intervals of time are much alike in shape.

A consideration of the six experiments described permits the following statements by way of summary. When discs of potato-tuber tissue approximately 1 x 15 mm. were soaked in buffer mixtures composed of H_3PO_4 and NaOH, secondary sodium citrate and NaOH, or potassium hydrogen phthalate and NaOH, the change in weight plotted against the H-ion concentration of the solutions expressed as pH produced a curve having a double maximum with a minimum between. With the H_3PO_4-NaOH series this minimum was located at a pH of 5.8–6.0; with the secondary

sodium citrate-NaOH series the minimum was found at pH 5.5–5.7; and with the potassium hydrogen phthalate-NaOH series the minimum was located at a pH of 6.2–6.25. In all three sets of the H_3PO_4-NaOH series, the least change in weight occurred in the solutions having a pH of 5.4. This was also true in one experiment with the secondary sodium citrate-NaOH buffer mixtures. The shapes of the curves were in each case much the same for all three weighings at 8, 12, and 24 hours, 12, 24, and 48 hours, or 6, 12, and 24 hours. The shape of the curve and the location of the minimum point for the turgid tissue at the end of 6 hours was the same as for the flaccid and presumably dead tissue at the end of 24 hours in the citrate and phthalate series.

The differences in weight between the potato in the solutions where the minimum weight was found and that of the potato in the solutions producing the maximum weight on the acid or alkaline sides of the minimum were not great. As shown in table 5, they ranged from 0.6 percent to 8.5 percent of the maximum total weight. This difference appears insignificant. Since, however, a large percentage of water must be present in resting tissue, it would appear that the difference expressed as percentage of gain rather than percentage of total weight might be of greater physiological importance. It is the small additional amount of water absorbed that is responsible for increased turgidity and for the phenomena which accompany it. If we consider the differences in weights from this standpoint they become more significant. Expressed as percentage of maximum gain, the majority of the differences were in the neighborhood of 25 percent. In other words, the gain of the potato tissue in solutions showing the minimum point was about 25 percent less than it was where the maximum gain was found on either side.

When the amount of the differences in gain in weight for the potato at the minimum and at the maximum on either side for the different buffer mixtures is considered, it would appear that in the phosphate series the more dilute the buffer mixtures of a given set the greater the difference. Thus, at the end of 12 hours the differences for the 0.1 M series and the two 0.01 M series between the acid maximum and the minimum were 0.29 g., as compared to 0.59 or 0.67 g. and 0.73 g. respectively. For the alkaline maximum and the minimum the difference was 0.32 g. compared to 0.64 g. and 0.41 g. With the citrate series the situation is complicated by the greater toxicity of the citrate ion.

Attention should also be called to the fact that it was only with the 0.002 M H_3PO_4-0.002 NaOH sets of buffer mixtures that gains as great as those found in distilled or redistilled water were observed.

A consideration of the change in reaction which the buffer mixtures showed as a result of contact with the potato indicates in general that up to pH 5.5–5.8 the solutions became more alkaline, while in solutions more alkaline than pH 5.5 or 6.1 they remained unchanged or became more acid. The results of the changes which occurred in the reaction of the buffer mixtures due to the presence of the potato are summarized in table 6.

Discussion

A consideration of the results of the foregoing experiments shows that the analogy between the absorption of water by such a protein as gelatin in solutions of different hydrogen-ion concentrations and the absorption of water by potato-tuber tissue under the same conditions is good if we assume that the isoelectric point of the potato tissue is in the vicinity of pH 6.0. In each case the water absorption by the potato tissue is at a minimum in the vicinity of pH 6.0 and increases in amount as we proceed toward greater alkalinity or greater acidity, passing through a maximum and then decreasing. This is what would be expected if the potato tissue acted as gelatin does and had an isoelectric point in the vicinity of pH 6.0. The shifts in the reaction of the buffer mixtures resulting from contact with the potato, to which attention was called in describing the individual experiments, are also what would be expected. As was pointed out in summarizing the experiments, those buffer mixtures in the vicinity of pH 6.0 (5.5–6.2) showed little or no change in reaction due to the presence of the potato. Those more acid showed changes in general toward greater alkalinity, and when those more alkaline than about pH 6.0 showed a change it was toward greater acidity. On the acid side of the isoelectric point a protein reacts with anions, with the result that the reaction of the solution in which the protein is placed should shift toward greater alkalinity. On the alkaline side of the isoelectric point it reacts with cations, resulting in a change of the reaction of the solution toward greater acidity. Part of the change in reaction in the alkaline solutions was probably due to the production of carbon dioxide by the potato tissue.

Two apparent difficulties prevent the analogy from being complete. The first of these is the fact that the position of the minimum point of water absorption, which should be the isoelectric point, is apparently affected by the acid radicle of the buffer mixture. Thus, with the phosphates it was apparently located at pH 5.8, with the citrate series at 5.5–5.7, and with the phthalate series at 6.2. These differences are apparently too great to be accounted for by errors in the determination of the hydrogen-ion concentration. It should be pointed out, however, that Michaelis and Rona (17) found that anions shifted the pH most favorable for precipitation of denatured albumin toward the acid side and that cations shifted it toward the alkaline side.

A second unexpected but interesting fact is that the dead tissue showed the same minimum for water absorption as the living tissue. This might suggest that it was not the living protoplasm with which we were dealing, but that either dead protoplasm killed in cutting the potato or non-living protein contained in the cells was concerned in both the living and the completely dead material. There is no complete evidence to demonstrate the truth of either of these possibilities. It would appear, however, that the differences obtained in the experiments with the dilute H_3PO_4-NaOH

buffer mixtures were too great to be accounted for by assuming that dead protoplasm or non-living protein was involved. Of course a comparatively small change in the isoelectric point with death would not be revealed by the methods used. Michaelis and Davidsohn (16) measured the isoelectric point of genuine albumin and of albumin denatured by heat by precipitation with sodium acetate. They found that the denaturing shifted the isoelectric point from pH 5.39 to pH 4.69.

MacDougal (14) has reported, in contradiction to earlier statements by the same author, that purified agar swells more in dilute HCl, pH 4.2, and dilute KOH, pH 11, than in water. The agar at 0.75 percent which is near the limit of its gelation at 15° C. had a pH of 6.5. These results indicate that pentosans may also show a double-maximum curve when their swelling is plotted against their pH. The confirmation of this possibility would permit an analogy to be drawn between the water absorption by potato and that by a pentosan. This analogy, with our present knowledge, would not hold for the changes in reaction which the potato produced in the buffer mixtures, nor would it explain the results obtained on the absorption of dyes reported later in this paper. Rosa (21), in some incomplete studies made in this laboratory, did not confirm MacDougal's results.

The Absorption of Dyes by Plant Tissue

It was pointed out in discussing the relation of the isoelectric point to the properties of gelatin that the amphoteric gelatin on the acid side of the isoelectric point forms salts with anions only, existing as gelatin chloride, gelatin sulfate, gelatin citrate, etc., while on the alkaline side of the isoelectric point it forms salts with bases existing as sodium gelatinate, potassium gelatinate, calcium gelatinate, etc. Since the colored radicles of acid dyes like eosin are in the acid radicle, gelatin of different hydrogen-ion concentrations takes up and retains the dye on the acid side of the isoelectric point and does not take it up or does not retain it on the alkaline side of the isoelectric point. With basic dyes like safranin, on the other hand, gelatin will be stained on the alkaline side of its isoelectric point (pH 4.7) and will not be stained on the acid side of pH 4.7. This method of determining the isoelectric point has been used by Loeb (13) for gelatin and by Thomas and Kelly (24) for the protein, collagen, of hide powder.

In applying the dye method to the investigation of the isoelectric point of plant tissue, potato-tuber tissue was used chiefly and found most satisfactory. A few experiments were also carried out with Spirogyra, leaves of Elodea, and sections of tomato and Kudzu stems. In general it was found that plant tissue which had been treated with buffer mixtures of different H-ion concentration responded to dyes as though it had an isoelectric point.

The potato used in these experiments was prepared as previously described for the experiments on water absorption. From 2 to 25 discs of

potato, 1 x 15 mm., were placed in tumblers in 200-cc. quantities of the buffer mixtures of different H-ion concentration. After periods varying from 1.5 to 5 hours, two discs from each solution were removed to a Syracuse watch crystal. They were covered with the dye, and after a few seconds' to 15 minutes' staining were removed, washed with redistilled water, and examined for relative intensity of staining. In some cases, after staining the pieces were washed with the original buffer mixtures. Water redistilled from alkaline and acid permanganate was used throughout in the preparation of the buffer mixtures and the solutions of the dyes.

Three sets of buffer mixtures were used, two composed of H_3PO_4 and NaOH and one of secondary sodium citrate and NaOH. Most of the experiments were performed using solutions obtained by mixing 0.1 M H_3PO_4 and 0.1 M NaOH and diluting 50 times. This was equivalent to mixing 0.002 M H_3PO_4 and 0.002 M NaOH. The original pH of these solutions as well as the pH of the solutions after 3 and 5 hours' contact with the potato are given in table 7. A set of buffer mixtures was also prepared by mixing 0.1 M H_3PO_4 and 0.1 M NaOH and diluting 5 times. This was equivalent to mixing 0.02 M H_3PO_4 and 0.02 M NaOH. The initial pH of the solutions used in this set and the pH of the solutions after standing in contact with the potato are also given in table 7. Buffer mixtures obtained by mixing 0.1 M secondary sodium citrate and 0.1 M NaOH and diluting 50 times were also used. This was equivalent to mixing 0.002 M secondary sodium citrate and 0.002 M sodium hydroxide. The original pH values of these buffer mixtures and the pH of the solutions after contact with the potato are given in table 7.

A consideration of the data there given confirms what was previously observed in the experiments on water absorption. The presence of the potato changed the pH of the solutions on the acid side of pH 5.8 toward greater alkalinity and on the alkaline side of 6.1 or 6.2 toward greater acidity.

The acid dyes used were orange G, eosin, Martius yellow, and acid fuchsin. The basic dyes were methylene blue, safranin, malachite green, and crystal violet.

Excellent results were secured with the acid dyes, eosin and Martius yellow, the potato responding as though its isoelectric point were located somewhat above pH 6.0.

The eosin was used at a concentration of 1–500 and the periods of staining varied from 3 to 10 minutes. To cite specific experiments:

Twenty slices of potato were soaked in the buffer solutions composed of 0.002 M H_3PO_4 and 0.002 M NaOH for 2 hours. Two slices from each buffer mixture were removed and stained in Syracuse watch crystals with 1–500 eosin for 3 minutes. When removed from the stain little difference in color was noted. They were washed in the watch crystals with 3 changes of redistilled water. The potato from solution 2, pH 2.85, was very red;

that from solutions 3, 4, 5, and 6, pH 4.9–6.1, less red than 2 but distinctly redder than that from 7, 8, 9, and 10, pH 6.95–7.85. The potato from the redistilled water, pH 6.5, was about like that from solution 6. The potato discs from solutions 3, 4, 5, and 6 were almost alike, with some tendency to greater intensity of staining in those from the more acid solutions. The potato discs from solutions 7, 8, 9, and 10 were almost alike. With 10 minutes' staining but more thorough washing, even better results were secured.

These results show that the acid dye eosin is absorbed and held by potato-tuber tissue from buffer mixtures whose average pH is 6.1 or less, and is held very weakly by potato discs from buffer mixtures of average pH 6.95 or above. In other words, the potato acted much as we should expect a protein to act with isoelectric point between pH 6.1 and pH 6.95.

Similar results were secured with the buffer mixtures made from 0.02 M H_3PO_4 and 0.02 M NaOH. After 3 hours' contact with the buffer mixtures, the potato was stained with eosin 1–500 for 5 minutes. The potato from solutions 2, 3, 4, 5, and 6, pH 2.0–5.6, was distinctly deeper red than that from solutions 7, 8, 9, and 10, pH 6.95–8.95 +. The line of separation appeared between 6 and 7.

With the citrate set of buffer mixtures, the potato was stained with 1–500 eosin for 10 minutes after 6 hours' treatment with the buffer mixtures. The intensity of color decreased from that of the potato from solution 2 to that of the potato from solution 10. The potato from the redistilled water stained about like that from solution 5. There was a fairly sharp line of separation between the potato from solution 5, pH 5.8, and that from solution 6, pH 6.2. However, the potato discs from solutions 6 and 7 were a little redder than those from 8, 9 and 10, which latter were about alike. This would place the isoelectric point either between pH 6.2 and pH 6.3 or between pH 6.3 and pH 6.5.

A number of experiments were also completed with Martius yellow, using the 0.002 M H_3PO_4–0.002 M NaOH buffer mixtures only. For example, the potato was treated with buffer mixtures composed of 0.002 M H_3PO_4 and 0.002 M NaOH for 2 hours. The pieces were removed and stained with the Martius yellow 1–2500 for 5 minutes. They showed little difference in intensity of staining at this time. They were washed with redistilled water several times and with the buffer mixtures for 20 minutes. An examination of the discs showed that the potato responded as would be expected if an isoelectric point existed between pH 6.1 and pH 6.95. The potato from the redistilled water stained somewhat less than any of the other pieces.

Orange G at a concentration of 1–500 for 1 minute and for 10 minutes was also used. Most of the stain, however, washed out, leaving only a little color in the pieces from the more acid solutions. A longer period of staining or a stronger solution of the dye might give more favorable results.

Acid fuchsin was another dye which did not yield as good results as eosin or Martius yellow. Used at a concentration of 1–4000 for 10 minutes, the staining was too weak to permit conclusions to be drawn. Used at a concentration of 1–500 for $2\frac{1}{2}$ minutes, on potato which had stood in the buffer mixtures of 0.002 M H_3PO_4 and 0.002 M NaOH about 6 hours, better results were secured. The pieces of potato did not stain uniformly with this dye, but irregularly, the vascular bundles taking the stain deeply. The potato discs from the acid solutions stained more heavily than those from the alkaline side, the line of separation appearing between solutions 6 and 7 as for eosin and Martius yellow. The potato from the redistilled water stained about like that from solution 6. The potato from solution 2 showed the heaviest staining.

The experiments with the basic dyes were considerably less satisfactory than those with the acid dyes. The potato from the different buffer mixtures, as well as that from the redistilled water, took the basic stains so uniformly that little difference in intensity of staining could be noted in the potato from the different buffer mixtures. This was at first very puzzling. Microscopic examination of the potato stained with safranin or crystal violet, however, showed that these dyes stained the starch grains and cell walls heavily. The hydrogen-ion concentration of the solutions in which the potato had stood apparently did not noticeably affect the intensity with which the starch combined with the dye. The intensity with which the starch and cell walls took up the dye covered up to a large extent any other effect present. With eosin the starch grains were not stained, and only the protoplasmic content of the cell took the dye to any considerable extent. This offers what appears to be a satisfactory explanation for the better results secured with the acid dyes. However, sufficient positive results were secured with safranin, crystal violet, and methylene blue to satisfy us that the basic dyes were taken up more strongly by potato from the solutions of pH 6.95 and above.

Confirmation of the conclusion that the basic dyes were taken up and held more strongly by the potato from the alkaline solutions was obtained by the way in which the stain was lost from the potato after staining. Thus, discs of potato, after standing 2 hours in the 0.002 M H_3PO_4–0.002 M NaOH buffer mixtures, were stained for 5 minutes with methylene blue 1–18000. The pieces were a uniform blue after staining. They were then laid in 15 cc. of the original buffer mixtures. After 2 hours the pieces from solutions 4, 5, and 6 were somewhat lighter in color than those from 7, 8 and 9. The difference, however, was not great. The buffer mixtures in which the stained potato had been washed were poured into test tubes, and the intensity of color was compared in the comparator. Those from the acid end were a deeper blue than those from the alkaline end. Thus 3, 4, and 5 combined were a much deeper blue than 7, 8, and 9; 4 and 5 were much bluer than 7 and 8. Little difference could be noted between 7 and 8 as

compared with 9 and 10. The stained potato was then replaced in 10-cc. quantities of the buffer mixtures and allowed to stand for 16 hours. When removed from the solution all pieces were colorless, in consequence of a reduction of the methylene blue. The exposure to the air and treatment with H_2O_2 developed the blue color. The pieces from the alkaline buffer mixtures were bluer than those from the acid buffer mixtures, although a sharp line of separation such as could be made in the case of the eosin-stained potato could not be made. The potato from solutions 2, 3, 4, and 5 was lighter blue than that from solutions 7, 8, 9, and 10, but the pieces from solution 6 were intermediate between those from 5 and 7.

Clearer-cut results with the basic dyes might be secured by weaker staining and with more thorough washing with the buffer mixtures after staining. The use of tissue with little or no starch and with large thin-walled cells well filled with protoplasm would also be advisable.

The response of dead potato was also compared with that of living potato, using the 0.002 M H_3PO_4–NaOH buffer mixtures. It was expected that a distinct difference would be found between the response of the dead and that of the living potato. The potato was killed by treatment with 50 percent alcohol. The living and the dead potato were treated with the same buffer mixtures for the same length of time and stained in the same dishes. No difference qualitatively was noticed between the dead and the living material. The dead potato, however, stained more deeply than the living potato.

Experiments were also carried out with Spirogyra with methylene blue and orange G. Difficulties at once arose, due to lack of uniformity of the material and the trouble of comparing the intensities of color in microscopic preparations. The Spirogyra was placed in buffer mixtures of 0.002 M H_3PO_4–0.002 M NaOH to which methylene blue at a concentration of 1–100,000 was added at once or after a period. The results of 5 experiments indicated that the greatest intensity of color was found in the Spirogyra from solutions 8, 9, and 10, that is, from solutions having a pH of 8.0 or more. Variations in the staining of duplicate lots and the difficulty of comparing the color intensity made accurate conclusions impossible. The same was true in experiments with orange G, although no difficulty was found in determining that the orange G was taken up more strongly from the acid solutions. Sprigs of Elodea, instead of Spirogyra, or sections of young tomato stems or Kudzu plants were also found unsatisfactory material. The small size of the sections and the presence of the natural green pigment made the difficulties too great to encourage experimentation with them.

To summarize briefly the results secured with the experiments on dyes: Discs of potato 1 x 15 mm. which were allowed to stand in 0.002 M H_3PO_4–0.002 M NaOH buffer mixtures of varying hydrogen-ion concentration for from 2 to 6 hours took up and retained acid dyes like eosin more strongly when the buffer mixtures had a pH of 6.1 or less than they did when the pH

of the buffer mixtures was 6.95 or more. Dead potato responded to a greater degree to staining, but qualitatively showed the same results. Using buffer mixtures of 0.002 M secondary sodium citrate and 0.002 M NaOH, the greater amount of dye was retained by those pieces from solutions of pH 6.2–6.3 or less while it was lost in washing by those pieces from solutions of pH 6.3–6.5 or greater. The acid dyes did not combine to any extent with the starch grains found in the cells of the potato-tuber tissue. With basic dyes the starch grains were stained, and pieces of potato from buffer mixtures of 0.002 M H_3PO_4 and 0.002 M NaOH stained rather uniformly. However, with care in staining and washing, it was demonstrated that basic dyes like safranin, crystal violet, and methylene blue were retained more strongly by potato from the solutions of pH 6.95 or greater than by those from solutions of pH 4.9–6.1.

Discussion

The analogy between the staining qualities of potato treated with different buffer mixtures and that of a protein like gelatin is good if we assume the isoelectric point of the potato-tuber tissue to be near pH 6.0. The potato-tuber cells, containing other materials like carbohydrates such as starch and pentosans and fat globules, and being surrounded by a cellulose wall, could not be expected to give such sharp and clear-cut results as the ash-free gelatin used by Loeb. The action of the basic dyes illustrates this point. It should be noted here that the position of what appears to be an isoelectric point as found by the method of water absorption agrees very well with that found by the dye method. In both cases it is located in the vicinity of pH 6.0. Attention should also be called to the fact that the death of the tissue, so far as these experiments gave evidence, did not affect the position of the isoelectric point.

General Discussion

The results which are reported in this paper on the absorption of water and on the absorption and retention of acid dyes by potato-tuber tissue can be explained by the assumption that an ampholyte, possibly a protein, plays the chief part in these processes and that its isoelectric point is in the vicinity of pH 6.0. The absorption of basic dyes by potato-tuber tissue is complicated by the fact that the starch, the cell walls, and possibly other constituents of the cell such as the pentosans, combine with the basic dyes at all the reactions involved. It is of course difficult to conceive of protoplasm as consisting of but one ampholyte with a single isoelectric point. The point to which we have called attention here may represent the resultant of the isoelectric points of several cell constituents.

While it is not considered that the hypothesis that potato-tuber tissue acts, in the processes indicated, like an ampholyte with an isoelectric point,

has been established by these experiments, it would appear very difficult to explain the phenomena which have been described on the basis of the conception of the cell as an osmotic chamber and of the theories of permeability of the plasma membrane which have been suggested. It would appear of extreme importance to reinvestigate the problems of water absorption and permeability, particularly with relation to salts and dyes, from this standpoint, bearing in mind that other constituents of the cell than proteins may react with dissolved material.[2]

Some substantiation of the conception that plant tissue may have an isoelectric point is afforded by the work of Osterhout on the conductivity of the tissue of Laminaria. Osterhout (19) found that alkali increased the conductivity of the tissue. Acid (20) was found to produce first a sharp decrease in conductivity, followed by a rapid increase. While no measurements were made on the hydrogen-ion concentrations of the solutions used, this result would be analogous to the changes which would take place in the conductivity of a protein which was originally in a solution more alkaline than the isoelectric point.[3] In the presence of alkali, the formation of greater and greater quantities of metal proteinates, accompanied by increased conductivity, would result. The addition of acid would first cause a decrease in the quantity of metal proteinates and probably produce an exosmosis of bases. When the isoelectric point was reached the minimum conductivity would be found. Further increase in acid would increase the amount of protein combined with anions and result in increased conductivity. It would appear advisable to reinvestigate the experiments which have been performed by Osterhout on the effect of salts and other conditions on the conductivity of plant tissue from the standpoint of the quantities of material which can conduct electricity rather than of the ease with which the electrolytes can move through the protoplasm.

The conception that protoplasm acts like an ampholyte with an isoelectric point in water absorption and in the absorption of solutes is one which, if substantiated by further investigation, would be of far-reaching importance. It would bear directly on the problem of water- and salt-absorption by plant cells, of the excretion of water by cells, the translocation of salts from one part of the plant to another, and conceivably on the rest period of plants in such cases as are apparently due to protoplasmic condition. (See Eckerson, 7.)

Whether we are correct or not in proposing the existence of an isoelectric point for plant tissue,[4] it would appear that the demonstration that there is a

[2] The analogy between the absorption of water by animal tissue and by proteins has been most completely presented by M. H. Fischer, in "Oedema and Nephritis," 3d ed.

[3] This has been suggested by W. O. Fenn. Similarity in the behavior of protoplasm and gelatine. Proc. Nat. Acad. Sci. 2: 537–543. 1916.

[4] From the effect of salts in acid and alkaline solutions on the absorption of dyes by Elodea, an isoelectric point of between p_H 3.8 and p_H 4.1 was proposed for the protoplasm of that plant by J. Endler. *See* Ueber den Durchtritt von Salzen durch das Protoplasma.

double-maximum curve with a minimum between for water absorption by plant tissue in solutions of different hydrogen-ion concentration offers a satisfactory explanation for the double-maximum curves found in the germination of spores and seeds, the growth of plants, and the infection of plants by fungi in media of different hydrogen-ion concentrations. The connection between water-absorption and spore germination or seed germination, and water-absorption and the growth of plants, is generally recognized. Recent evidence seems to indicate that the infection of plants by fungi which enter through the unpierced epidermis is due to a pressure process. A minimum water absorption would mean a minimum pressure and a minimum infection.

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STUDIES OF *LYTHRUM SALICARIA* I. THE EFFICIENCY OF SELF-POLLINATION

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The conditions that exist in species with trimorphic flowers permit the investigation of the problems regarding the nature of sex-differentiation and the degrees of compatibility between male and female organs under very favorable circumstances. In these forms the morphological adaptations for cross-pollination are often decidedly correlated with physiological incompatibilities which make cross-fertilization more certain by excluding the functioning of the chance self-pollinations which occur.

In general it is to be recognized that sexual fusions are favored by similarity of the gametes both in genetic constitution and in immediate origin, and that such conditions as trimorphism and self-incompatibility are to be regarded as, in a high degree, secondary and acquired. While these conditions secure the advantages of bringing together gametes of different origins and in greater or less degree of different genetic constitution, they decidedly limit and restrict free fertilization and full productivity.

The combination of morphological trimorphism with physiological incompatibilities as seen in species like *Lythrum Salicaria* may well be regarded as the highest degree of specialization in sex-determination and fertilization that exists in flowering plants. For this species there is the obvious morphological differentiation giving three lengths of pistils and three sets of stamens of lengths corresponding to those of the pistils, with the stamens bearing pollen differentiated as to size, color, and nature of the reserve food material in storage. For the individual, the flower of any particular plant has a pistil of one of the three lengths and a set of stamens for each of the other two lengths. This gives differentiation of forms as such, and in the single plant there is the differentiation that gives two kinds of stamens. Furthermore, this morphological differentiation is decidedly correlated with physiological differentiation. The noteworthy researches of Darwin (1865, 1877) showed that there is marked or even complete sterility to (1) self-pollinations, to (2) intra-form cross-pollinations, and to (3) the inter-form cross-pollinations that are illegitimate (*i.e.*, those that involve different lengths of pistil and stamen). Seed-production was hence found to be more or less limited to legitimate pollinations, which are necessarily crosses.

The specializations in these forms allow no doubts as to their significance such as have often been raised in regard to the colors of entomophilous flowers, for here the adaptations are morphological and depend directly

on the agency of insects for their effectiveness in pollination. The relative lengths of the different sets of stamens and of the pistils are unquestionably provisions favoring crossing.

It is, however, obvious that such differentiations are not fundamental expressions of sexual antithesis, since they are all reciprocal in any pair of flowers. Any two plants of any two forms are cross-compatible or cross-incompatible according to whether the cross is legitimate or illegitimate. The differentiations, at least in respect to maleness, that in dimorphic plants are seen in individuals as such and which appear to have definite genetic value are here seen equally well in the two sets of stamens in a single flower. All this emphasizes the fact that the conditions are secondary and acquired in contrast to the more primitive condition of homomorphism and a more general compatibility of gametes.

The generally accepted view has been that the differentiations in this species are well established and very stable. The tendency has been to emphasize, as did Darwin, the evidence that here there is adaptation favoring crossing, and to pass the evidence, which has to some extent been noted, that the adaptations are incomplete. It is to be recognized that such evidence has a very direct bearing on questions of the origin of trimorphism, of the nature of sex-differentiations, and of whether there is still opportunity for further selection in the species either toward greater or toward less restriction of fertilization. It is evidence along these lines that the writer wishes to present in reports, of which this is the first, of investigations with the species.

The Efficiency of Self-pollination for Plants Grown in Isolation

The writer's studies of *Lythrum Salicaria* were begun in 1917 in testing the self-compatibility of plants grown in isolation from other plants of the same or of related species. On such a plant hundreds of flowers open daily during a rather extended period of time and insect visitors can go from flower to flower, but with no chance, if the isolation is complete, of bringing pollen from other plants of *Lythrum Salicaria*. A large number of flowers are thus involved in the chance for self-fertilization (including here autogamy and geitonogamy), and the results can be obtained for the entire period of bloom. This test does not, however, determine the relative fertility of a plant to pollen of its two sets of stamens, nor does it reveal the need or the efficiency of particular species of insects in the self-pollination of the various forms, which may indeed give results that are highly variable from season to season or from year to year or according to location. However, if seed is produced there is positive evidence of self-compatibility, and the negative results may be tested further by controlled pollinations.

Short-styled Plants Grown in Isolation. Two large, well-developed plants several years old were dug from a mixed population growing at the New York Botanical Garden. One (*S no. 2*) was grown in the garden of the

Department of Botany at Columbia University, under the observation of Professor R. A. Harper, and one (*S no. 1*) at the University of Missouri under the care of Professor G. M. Reed. Both plants made vigorous growth and bloomed profusely, but neither plant produced a single capsule during the entire period of bloom of the season of 1917. In 1918 the plant at Missouri suffered severely from drought, and its failure to set any seed that year is not to be considered as adequate evidence of complete self-incompatibility.

The short-styled plant at Columbia University thrived and in the course of its season of bloom in 1918 produced 17 pods. Ten of these contained only mere rudiments of seeds, two contained one good seed (plump and apparently fully developed) each, three had two good seeds each, one had seven seeds, and one had eight. Sixteen of these 23 seeds germinated. In the following year (1919) this plant bloomed more abundantly than in the previous year and produced at least 5000 flowers, and during the entire season 161 pods matured. The seeds in 100 of these pods were counted; the number per pod ranged to 116, and the average was 23.67 (see table 1). This plant grew poorly in 1920, when its roots were separated to make two plants. In 1921 these thrived, and there was abundant bloom but only about 25 pods were produced. The irregular pod production by this plant may involve one or more of several conditions; possibly in 1919 insects may have brought pollen from a distance from plants of this species growing in city parks, or the irregularity may involve the presence or absence of certain insects that are most efficient in causing self-pollination.

Long-styled Plants Grown in Isolation. A long-styled plant (*L no. 1*) was grown at Baraboo, Wisconsin, under the care of Mr. William Toole, Sr., a well known pansy specialist. Plants of the variety *rosea* were also growing in the nursery at some distance away, so that some of the seed produced by the plant *L no. 1* may have been due to cross-pollination by insects. But another long-styled plant (*L no. 2*) was grown in what was certain isolation at Pleasantville, N. Y., under the care of Dr. M. A. Howe. Capsules were produced by both these plants. Of the 65 capsules on *L no. 1* in 1917, 16 contained no seeds, 17 contained one seed each, and the highest number of seeds in any capsule was 17. In 1917 a total of 53 capsules matured on the plant *L no. 2*. As shown in table 1, the number of seeds per capsule for this plant was also low, although all but three of the capsules contained some fully matured seeds.

In 1918 both plants produced capsules quite as in 1917, but the capsules on *L no. 2* were somewhat more numerous than in the previous year though still constituting a small proportion of the entire number of flowers.

The capsules produced by these two plants were distributed irregularly but rather indiscriminately throughout the flowering branches. Self-fertilization appeared to be effected with the same frequency and efficiency throughout the flowering period. No very decided seasonal differences appeared in respect to the proportional number of flowers which matured

TABLE I. *Self-compatibility in Lythrum Salicaria to insect-pollination, and cases typical for the results obtained for controlled hand-pollinations*

	Number of Flowers Pollinated	Number of Pods Produced	Number of Pods used in Seed Counts	Distribution of Seed per Pod. Class Groups 1 to 10												Average Seed for Pods with Seed
				0	-10	-20	-30	-40	-50	-60	-70	-80	-90	-100	-110	-120	-130	
Isolation, Insect-pollination																		
S. no. 1.....	?	0																
S. no. 2. (1918)...	?	17	17	10	7													3.28
S. no. 2. (1919)...	?	161	100	0	34	30	16	4	5	4	0	2	1	2	0	2		23.67
L. no. 1.....	?	65	65	16	47	2												2.96
L. no. 2.....	?	53	53	3	47	3												4.85
M. no. 2.....	?	3000	300	14	202	48	8	7	6	1	5	1	1	1	2	3	1	8.91
M. no. 1. (1919) upper ⅓	?	many	71	7	43	12	3	2	1	0	2	0	1					
M. no. 1. (1919) middle ⅓	?	few	31	3	16	3	0	3	2	1	2	0	0	0	0	1		
M. no. 1. (1919) lower ⅓	?	few	40	6	20	3	1	2	2	0	1	1	0	1	2	1		
" total.....	?	1500	142	16	79	18	4	7	5	1	5	1	1	1	2	2		18.25
Hand-pollination																		
M. no. 1. (1919)..	233	134	134	1	28	29	19	20	14	6	5	8	3	0	0	1		24.61
M. 5–1 no. 13.....	103	0																
M. 3 no. 7.....	48	8	8	1	5	2												7.00
M. 1 no. 48.....	35	6	6	0	1	2	2	1										20.33
M. 1 no. 31.....	33	4	4	0	0	2	1	1										25.00
M. 1 no. 56.....	60	9	9	0	0	4	3	0	1	1								28.33
M. 5–1 no. 25.....	32	9	9	0	0	0	2	1	0	3	2	1						50.88
M. 5–1 no. 35.....	32	5	5	0	0	1	1	0	0	2	0	0	1					49.50
M. 1 no. 30.....	37	9	9	0	0	0	2	0	2	1	1	0	1	2				59.66
M. 1 no. 57.....	40	44	44	0	1	7	3	2	7	3	3	12	5	1				63.15
L. 4 no. 2.....	55	2	2	0	2													6.00
L. 1 no. 66.....	68	2	2	0	2													6.00
L. 2 no. 18.....	50	7	7	0	6	1												7.42
L. 1 no. 8.....	49	22	22	0	11	10	1											8.90
L. 5–5 no. 38.....	24	6	6	0	2	1	2	1										20.00
S. 5 no. 5.....	14	4	4	0	1	2	1											14.00

capsules that could be referred definitely to differences in insect visitations or to the constitution of the plants.

Mid-styled Plants Grown in Isolation. A plant of this form (*M no. 2*) was sent to Mr. H. L. Skavlem, who grew it at Carcajou Point, Lake Koshkonong, Wisconsin. Mr. Skavlem states that by the middle of July this plant was "over four feet high with an abundance of bloom which continued for about six weeks." The plant bloomed from July 14, 1918, until the first week in September. There were about 35 well-developed main flower spikes ranging from 3 to 14 inches in length, and the total number of flowers produced was estimated at 8,000. This description would apply as well to any well-developed plant of any of the three forms.

This mid-styled plant was decidedly self-compatible in this isolation test. At least 3,000 capsules matured on it in 1917. A total of 300 capsules were examined and counts were made of the seeds present, with

results as summarized in table 1. The number of seeds per capsule ranged to 121; twenty-eight capsules had more than 30 seeds each, but 202 capsules contained less than 11 seeds per capsule, and 14 contained only rudiments of seeds. The average number of seeds per pod for those that had good seeds was 8.91. In 1918 the self-compatibility of this plant was quite the same as in 1917.

In making the counts of seeds in 300 capsules (1917 crop) of this plant, position was taken into account. Each branch involved in the counts was divided into three sections of equal length, here designated as the lower third, middle third, and upper third. In the lower third of all branches there were 10 capsules with more than 30 seeds each. It is clear that the average number of seeds per capsule was lower toward the top of the branches of this plant, but smaller capsules and fewer seeds per capsule are, as a rule, to be expected toward the apex. Otherwise the plant was rather uniformly highly self-compatible, and capsules with seeds were produced in a considerable proportion of the flowers subjected to open pollination throughout the entire period of bloom.

Another mid-styled plant (*M no. 1*) was grown in my own home garden. This plant made a vigorous growth and produced in 1917 at least 6,000 flowers. At the close of the season it was found that relatively few of the flowers produced pods during the first two thirds of the period of bloom, but that later nearly every flower produced a pod.

In 1918 it was planned to test experimentally the behavior of this plant, and especially to determine if the marked difference in production of fruit involved changes in the innate ability of the plant to produce fruit or indicated merely some difference in insect-pollination. A large, well-developed long-styled plant was planted by its side. The two began blooming only one day apart. The long-styled plant was allowed to bloom, thus affording opportunity for free cross-pollinations by insects between the two, until the 17th of August, when the long-styled plant was cut down. During the time that both were in bloom nearly all flowers that opened on the mid-styled plant developed fine pods, showing that the feeble production of pods during the early part of bloom as observed in the previous year when the plant was in isolation was not due to an impotence of the pistils. During the rest of the season its performance was quite as in the previous year.

In 1919 the long-styled plant was kept cut down so that no flowers were produced by it, and a series of guarded self-pollinations were made on the plant *M no. 1*. Branches were enclosed in glassine paper bags. Whenever pollen from short stamens was used for pollinations, the flowers to be thus pollinated were opened early in the morning and the long stamens were removed, and then pollen from the short stamens was used later in the day when the anthers were dehiscing.

Legitimate cross-pollinations, using pollen of flowers brought in vials from the experimental plots over a mile distant, were made on 22 flowers

during the first 18 days of bloom. The plant was in bloom 55 days. The results obtained for selfing are summarized in table 1 and are shown in more detail in table 2, in which, to facilitate ready comparison, the data are compiled for three periods.

TABLE 2. *Record for controlled pollinations of mid-styled plant no. 1 in summer of 1919*

	1st to 18th Day	19th to 36th Day	37th to 55th Day
Selfed with pollen of long stamens			
Failures—no pods	70	35	9
Pods produced	18	46	70
Seeds per pod—range and average	(0 to 117) av. 34	(2 to 81) av. 32	(3 to 82) av. 32
Selfed with pollen of short stamens			
Failures	33	57	47
Pods	1	1	1
Seeds per pod	37	10	1
Legitimate cross-pollination			
Failures	2		
Pods	20		
Seed-range and average	(12 to 176) av. 98		

Of the 140 flowers hand-pollinated with pollen from short stamens, only three produced pods yielding 1, 10, and 37 seeds respectively, but these may have been due to chance pollination with pollen from the long stamens at the time of their removal or with pollen of other flowers which were enclosed in the same bag. The results indicate that the plant remained decidedly if not completely self-incompatible to pollen of its own short stamens throughout the entire period of bloom.

When pollen of long stamens was used in hand-pollinations, the proportion of pods produced increased as the season advanced, and during the last 18 days of bloom there were but 9 failures out of 79 flowers pollinated. The results show conclusively that the self-compatibility in this plant involves fertilizations from the pollen of long stamens, and also that this compatibility actually increases toward the end of the period of bloom. The increase in compatibility affects, however, the number of pods that are formed rather than the number of seeds in a pod. The average number of seeds in the pods that were produced was almost the same for all periods, but the highest number of seeds in any pod was obtained during the first 18 days of bloom.

The 22 legitimate crosses made during the first 18 days gave 20 pods with seeds ranging in number from 12 to 176, with an average of 98 seeds per capsule. These results show conclusively, as do those of open cross-pollinations during the early part of the period of bloom in the previous year, that the pistils of the plant are highly potent during the period of marked self-incompatibility. The decided change in fruit-production s

hence due to a change in the physiological relations between pistils and the pollen of long stamens. Pollen from both short stamens and long stamens was examined at frequent intervals throughout the entire period of bloom; there was very little abortion, the pollen was successfully germinated in artificial media, and the use of such pollen in legitimate crosses on several dates during the first 30 days of bloom was almost invariably highly effective in pod- and seed-production. There were in this plant no noticeable evidences of impotence of stamens and anthers, such as are to be seen in some plants of this species.

The branches left to open-pollination produced pods quite as in the year 1917. Several of the main branches were selected and divided into thirds, and seeds in all pods in the lower two thirds were counted; then an equal number of pods from the many pods in the uppermost third were taken at random for counts, the entire number, 142, being as near the number of pods secured from the selfing by hand in which the pollen of long stamens was used as was possible. As shown in table 1, the range for number of seeds per pod and the average were higher for the lower two thirds than in the last third, showing that the change in compatibility involves number of pods formed rather than number of seeds per pod. Comparison shows that the controlled pollinations in which pollen from long stamens was used were somewhat more successful than free open-pollinations, when judged by the average number of seeds produced.

A third mid-styled plant (*M no. 3*) was grown in isolation in the New York Botanical Garden at a distance of about one mile from the location of the plant *M no. 1*. This plant proved to be decidedly less self-compatible to open self-pollination than were *M no. 1* and *M no. 2*, but, as in *M no. 2*, there was quite the same proportion of pods produced throughout the entire season of bloom, no change in compatibility being evident as observed in the plant *M no. 1*.

Summary. In these tests seeds were obtained to illegitimate self-pollination of plants of all three forms. Mid-styled plants were most highly self-compatible in respect to the number of pods produced. The pods found were distributed irregularly over the entire period of bloom except for one plant (*M no. 1*), in which it was found that there was an actual change in the degree of self-compatibility to pollen from its own stamens. The results from year to year have been very uniform for all plants, except for the plant *S no. 2*. Its feeble production of pods in 1918, followed by the production of 161 pods in 1919, suggests that the plant is rather strongly self-compatible provided insects make the proper pollinations. It may readily be conceded that the kinds of insects that can most efficiently self-pollinate long-styled and mid-styled plants are not the ones which best self the short-styled plants.

Self-pollination does not appear to be uniformly as efficient in seed-production as are certain legitimate cross-pollinations, at least for the one

mid-styled plant *M no. 1* (see table 2). Of the 22 flowers on it that were crossed during the first 18 days of bloom there were only two failures, the highest number of seeds for a capsule being 176 and the average 98. Whether such pollination would be more effective than selfing during the last part of the period of bloom was not tested.

Self-compatibility Tests by the Bagging Method

A total of about 600 plants have been grown in pedigreed cultures from seed. A considerable number of these have been tested for self-compatibility in the following manner: branches were enclosed in glassine paper bags, and pollinations of flowers opening within were made from day to day. Long-styled plants and short-styled plants were selfed by using the pollen from mid-length stamens, and the mid-styled by use of the pollen of long stamens. In making pollinations, stamens with dehiscing anthers were removed with sterile tweezers and brushed on pistils, leaving an abundance of pollen. In cases of pseudo-proterogyny the fully protruding pistils of partially opened flowers were likewise treated along with pistils of more mature flowers. It appears that in the decidedly pseudo-proterogynous flowers the pistils protrude long before they are receptive to any kind of fertilization, and that highest seed production in compatible fertilizations occurs when pollination is made at or about the time that petals open and pollen is shed. On plants two or more years old, a total of as many as 500 or more flowers were often thus pollinated. On plants in the first year of growth from seed the number thus selfed was often much less.

These tests are undoubtedly less adequate than tests in isolation for revealing feeble grades of self-compatibility and in showing such changes in self-compatibility as are seen in the plant *M no. 1*, but hand-pollinations make certain that pollen in abundance is applied to the stigmas at the time when they are judged to be most receptive.

The general results summarized for each form without reference to lines of descent are as follows:

	Fully Self-incompatible	Feebly Self-compatible	Medium Self-compatible	Highly Self-compatible
Mid-styled plants.....	64	20	21	7
Long-styled plants.....	83	14	0	0
Short-styled plants....	22	1	0	0

An attempt has been made to grade the plants according to whether the self-compatibility is feeble, medium, or strong, the judgment being based on the proportion of selfed flowers that gave pods and the number of seeds produced. Results typical for various grades are given in table 1. The tests made show that many plants of the species are without doubt entirely self-incompatible (*M 5–1 no. 13* in table 1 for example), and that others are

highly self-compatible (*M 1 no. 57*), with various intervening grades the grouping of which is neither definite nor accurate.

In these tests mid-styled plants have been more highly self-compatible than plants of the other forms. This is true both in relative numbers that produce fruit to selfing, and in the range to higher grades of fertility. On one plant, *M 1 no. 57*, every flower that was selfed produced a pod, and four other pods were produced in other flowers that spontaneously selfed while enclosed in a glassine bag.

Of the 97 long-styled plants tested in hand-pollinations, only 14 produced pods, and not one gave over 30 seeds in any pod. In all of these the self-compatibility was apparently of a weak grade.

Twenty-three short-styled plants were tested, and only one produced seeds.

The results obtained in the controlled self-pollinations with these plants agree in general with those obtained in isolation tests. A rather large proportion of mid-styled plants are self-compatible in some degree, and nearly half of the plants of this form produced pods containing viable seeds to selfing, and a few were highly self-compatible. There has been no difficulty in finding mid-styled plants to use as parents of self-fertilized lines of progeny. Relatively few long-styled plants produced pods to selfing, and in all such plants the self-compatibility was feeble, few pods being produced and these having few good seeds. Short-styled plants have as a class been decidedly self-incompatible, and of the seedlings tested only one has produced seeds to selfing. The high seed-production seen in the plant *S no. 1* in 1919 was not duplicated by the plant in 1918 nor in 1920 and 1921. There has not been opportunity to test this plant by controlled hand-pollinations as the plants grown from seed have been tested.

Summary

1. Many plants of *Lythrum Salicaria* are capable of producing capsules and viable seeds to illegitimate self-pollination brought about either by controlled hand-pollination or by insect-pollination in the field. The capacity for self-fertilization still lingers strongly in the species.

2. The proportion of self-compatible plants is greatest in the mid-styled plants, in which also the highest grades of self-compatibility are to be seen. Long-styled plants are, as a class, less self-compatible, and the short-styled plants are still less so. The three forms appear to differ in the capacity for self-compatibility.

3. There are wide variations in the degree of self-compatibility. In the most highly self-compatible form, the mid-styled, there are all gradations between complete self-incompatibility and the highest grade of self-compatibility.

4. The variations in the physiological condition of the sex organs, as

exhibited in selfing, suggests that wide variations may likewise be expected for crossings even for those that are legitimate.

5. One noticeable case of end-bloom self-compatibility was found. This was in a mid-styled plant and involved only fertilizations from pollen of the set of long stamens.

6. The physiological relations of the sex organs in plants of this trimorphic species exhibit quite the same range of variations as are seen in many homomorphic species.

Conclusion

For the species *Lythrum Salicaria* the evidence of wide variation in the degree of self-incompatibility is definite. The physiological differentiations of the sex organs are incompletely correlated with the apparent structural adaptations for cross-pollination; they are not fixed, constant, and fully achieved either in expression or in heredity, but are fluctuating and intergrading. They still present opportunity for further selection either toward greater or toward less restriction of fertilization.

The persistence of self-compatibility in various degrees of expression, and the apparent difference in respect to self-compatibility seen among the various forms, present strong evidence that self-compatibility was the antecedent condition in the species out of which the present complex of sex relations is still evolving, just as the sets of styles and stamens of different lengths have been developed out of an original homomorphic species.

New York Botanical Garden

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CHROMOSOME BEHAVIOR IN *ACER PLATANOIDES* L.

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That chromosomes may be observed as definite bodies at nearly all stages in the life of a cell has been shown by various workers. Rosenberg (1904) and Overton (1905) were among the first to direct attention to the individual chromosomes in the resting nuclei in plant cells as definite chromatin bodies. In addition, Overton (1909) has figured these chromatin masses, "prochromosomes" as he termed them, in the various stages of the reduction division in the pollen mother cells of Thalictrum, Calycanthus, and Richardia. He maintains that these prochromosomes are in pairs in the resting nucleus of the mother cell; that in synapsis a continuous bivalent spirem exists in which the paired prochromosomes are arranged side by side at more or less regular intervals, and that they remain thus associated in parallel pairs until they separate at metaphase. Evidently these observations and conclusions of Overton are in support of the Grégoire school which maintains that there is a side-by-side pairing of the chromosomes in synapsis.

Digby (1919) has brought forward some corroborative evidence in support of the view of Farmer and his followers that the splitting of the spirem preceding synapsis is the same as the splitting in the telophases of the sporogenous mitosis, and that there is an end-to-end pairing of the chromosomes in synapsis rather than a side-by-side pairing. The difference between these two views of how the chromosomes pair appears to be due largely to differences in interpretation of stages associated with synapsis. Evidence which has a direct bearing upon this problem has been found in the study of the heterotypic division of the pollen mother cells of the Norway maple, *Acer platanoides*; in this plant the behavior of the individual chromosomes may be followed with considerable clearness through synapsis. Cardiff studied this species, but his findings differ in several particulars from those of the writer, so that a reconsideration of this form seems justified.

METHODS

In the preparation of the material for this study, the flower buds of *Acer platanoides* were collected in the early spring when the buds first began to enlarge. After removing the scales, the buds were dipped in 50 percent alcohol and then fixed in either Flemming's medium solution or in Bouin's solution. Dipping in alcohol caused the flower cluster to sink at once when placed in the fixing fluid and resulted in more uniform fixation of the

material. Bouin's solution gave the better results in fixation, causing less shrinkage of the cytoplasm and less clumping of the chromosomes. The best results in staining were obtained by using the safranin, gentian violet, and orange G combination. The sections were first bleached for twelve hours in full-strength commercial hydrogen peroxide; following this they were stained one minute in a saturated, 50-percent alcoholic solution of safranin; five minutes in a similar solution of gentian violet; a few seconds in an aqueous solution of orange G; the slide was then flooded with absolute alcohol followed with clove oil, which was allowed to remain until the stains had been extracted so as to give the desired differentiation. This method gave a large number of excellent preparations showing marked differentiation between the nucleolus, linin, and chromatin contents of the cell. Heidenhain's iron-alum haematoxylin stain was used as a supplementary one, but it did not give the satisfactory differentiation of the triple stain.

Observations

Attention was given primarily to the nuclear changes in the pollen mother cell during the stages of its first division. The somatic cells of the flower stalk and of the roots were examined as well as several megaspore mother cells. The pollen mother cells were exceptionally good for study in that each flower bud frequently showed several stages of development, a single loculus of an anther often including a progressive series of stages from one end to the other.

In the earliest stage to be found in the pollen mother cell, the chromatin-staining material exists as several well-defined bodies, varying somewhat in size, which are distributed about the periphery of the nucleus; a few of these bodies, however, appear to be scattered through the nucleus, and some are usually lying against the nucleolus (Pl. XXXI, fig. 1). Not infrequently two chromatin bodies are found lying near each other; because of the similarity in size and position one is led to believe that they belong together as a pair. The distance between the individuals of a pair varies; sometimes they are nearly in contact, while in other pairs they are separated by a space at least equal to their own width. Some of the bodies do not appear at first to be paired, yet in view of their subsequent behavior it is not difficult to associate practically each one with a nearby mate. It is not easy when one sees so many conspicuous cases of pairing in the nuclei of this stage to believe that these are merely chance positions, as interpreted by Mottier in *Acer negundo*. The number of these chromatin bodies is probably twenty-six, as determined by an examination of numerous complete nuclei.

The linin occurs as very fine, faintly stained threads, usually forming an irregular netlike structure to which the chromatin bodies appear to be attached. Occasionally enlarged nodes will be observed on the linin where two threads cross or come together; these nodes are distinguished from the

chromatin bodies by their faint staining reaction and their irregularity of outline.

Attached to the nucleolus is usually one, rarely two, bud-like structures which take the safranin stain like the nucleolus; sometimes they are found disconnected from the nucleolus. They vary somewhat in size but are usually somewhat larger than the chromatin bodies, from which they are further distinguished because of their reaction to stains and because of their more globular shape (fig. 2).

The first evidence of growth on the part of the mother cell is an increase in size of the nucleus as well as of the entire cell. The linin stains more readily, and many of the chromatin bodies are more conspicuously arranged in pairs. At no stage could I be sure that the individuals of a pair are connected by linin. The cytoplasm becomes somewhat less dense as the cell enlarges, but the changes are so slight that one would not be justified in using them as criteria for determining the stages of development of the nucleus as did Cardiff in his study of this species.

The next apparent change is a more conspicuous linin which becomes pulled away from the periphery of the nucleus in several places and collected to form the synaptic knot (fig. 4); usually, even at the most contracted phase of synapsis, there are a few free threads extending outside of the knot, some of which reach to the nuclear membrane (fig. 5). There is no conspicuous paralleling of the threads during the pre-synaptic stages nor in synapsis; only occasionally will two threads be seen parallel to each other. The chromatin bodies remain distinct throughout the early stages of synapsis; as the linin becomes more contracted into a knot, the individuals of a pair come closer together until they are in contact with each other. Throughout these stages there are always some chromatin bodies lying against the nucleolus, and frequently a group of four will be found rather close together.

At about the period of maximum contraction of the linin the chromatin bodies appear to lengthen out; the individual members of a pair seem to flow out in opposite directions, indicating an end-to-end union of the two; in this manner short, thick threads are formed which take the chromatin stain (figs. 7–9). In a single nucleus some of the chromatin material may be seen as definite paired bodies, while some appears as short, thick threads which fade into the less deeply stained linin. In a few cases one or more chromatin threads were found outside of the knot with only one end entering it; upon these threads were darker-stained portions indicating the position of the pair of chromatin bodies. No evidence was found to indicate that two fine parallel threads unite to form a single large one.

The synaptic knot becomes more and more converted into thick threads by the flowing out of the chromatin material until there appear to be several of these thick threads, more or less massed, with some free ends extending out from the knot (fig. 11). It is possible that these threads are

all joined together into a single long, tangled one, but after an examination of numerous cells of this stage, such a conclusion seems hardly warranted. These thickened threads soon become less tangled, usually with one end extending into a clump at one side of the nucleolus (Pl. XXXII, fig. 13); this stage resembles that figured by some workers as the second contraction stage. Commonly one or more individual threads do not extend into the clump. Many of the threads at about this stage show a lighter longitudinal area indicating a longitudinal splitting; the splitting becomes so nearly complete in some cases as to separate the two halves of the thread for at least part of its length. These threads often contain as many as four or five deeper-staining places somewhat varied in size; on the split threads these appear as paired, equal-sized bodies (fig. 13). At about this stage the cytoplasm becomes drawn away from the cell wall and more condensed about the nucleus.

The next apparent change in the cell is in the general distribution of the threads throughout the nucleus; many of the threads are bent or looped (fig. 14). As development proceeds, the threads become shorter, usually more ragged, and several of them soon become shaped like a *C*, a *U*, or an *O*; at this stage the split has disappeared (figs. 15–17). One cannot be certain that each one of the threads folds over on itself; in fact, after studying numerous nuclei of this stage and after considering the subsequent stages in the separation of the individuals of a pair, I am of the opinion that some threads do fold over and some do not. There is no direct evidence that the bend in the thread occurs where the two chromatin bodies of a pair unite, but it is reasonable to suppose that such is the case. These bent threads become shorter, and some of them at least segment at the bend; contraction and condensation of the chromosomes continue while the membrane of the nucleus disappears (figs. 18, 19). Some of the threads become condensed into chromosomes before others; this fact may be associated possibly with the difference in size of the chromosomes which one observes at metaphase. This irregularity, or variation, in development is also evident as the chromosomes are separated at metaphase, some individuals of a pair being entirely separated while others are intact. As the members of a pair are being separated, some of the elongated ones show rather clearly that they are lying side by side in the spindle. There is no evidence of splitting of the individual members of the bivalents during these stages. When the nuclear membrane disappears, the chromosomes become collected about the nucleolus, which in turn becomes elongated and irregular, gradually disappearing as the chromosomes become arranged on the spindle.

As the univalent chromosomes move toward the poles they appear to become more condensed, forming short, thick lumps; there is some indication of a splitting of the univalents during this stage, but the evidence is not convincing. At the poles the chromosomes retain their individuality; they become somewhat larger, irregular in shape, and some, at least, have

one or more threads attached to them (fig. 21). As the daughter nuclei increase in size, one or more nucleolar bodies appear in connection with some of the chromosomes. In the resting stage of the nucleus the chromosomes become somewhat more condensed, and there is usually but a single nucleolus present (figs. 22 *A*, 22 *B*). This resting stage appears to be of short duration. The chromosomes become more angular during the succeeding prophase stages and appear to be split about the time that they are drawn toward the equatorial plate; several of them are in contact with the nucleolus, which in turn becomes irregular and extended. In some cases the two spindles are arranged in the same plane, but more usually they are at right angles to each other. The further stages of division of the daughter nuclei appear to be like those of the heterotypic division. The tetrad or granddaughter nuclei show clearly the thirteen chromosomes scattered about in the nucleus (fig. 23).

In the resting stage of the vegetative cells the chromatin occurs as definite chromatin-staining bodies; there is no conspicuous pairing of these bodies in this stage, but when the nucleus is undergoing the late prophase stages in division, the association of chromosomes of similar shape and size does become apparent (fig. 25), although they were never found joined together; this same condition of pairing was observed in the metaphase stage (fig. 24).

In the megaspore-mother-cell nucleus, several prophase stages were observed including synapsis, but all presented the same phenomena as those observed in similar stages of the pollen mother cell.

Discussion

Cardiff in his work on *Acer platanoides* called attention to the chromatin in the resting nucleus of the mother cell as collected in small bodies at the periphery of the nucleus, but he did not consider their number. Although one cannot be entirely certain, yet I am convinced that the number corresponds to the number of chromosomes observed in the division stages of the vegetative cells. These chromatin masses are doubtless a phase in the life history of the chromosomes, and correspond to the prochromosomes of Overton. In my earlier work on *Acer negundo* similar chromatin bodies were observed, but no attention was given to the number present; a reexamination of these stages as well as of later ones has led me to conclude that the phenomena of chromosome-formation in the two maples are essentially the same and that certain stages in the preceding work were misinterpreted.

The bud-like structures on the nucleolus were noted by Cardiff and figured both by myself and by Mottier in *Acer negundo*. There is evidence that these break off from the nucleolus during the growth period of the cell, since occasionally one or more are found lying free in the nucleus; this fact may be determined in nuclei well stained with the triple stain, in which the nucleolus and these buds stain with the safranin and the chromatin bodies

stain with the gentian-violet. These free buds disappear as development proceeds. As to the significance of this behavior there is no direct evidence; it may possibly be an early fragmentation of the nucleolus, or it may be associated with chromatin anabolism; the latter is perhaps the more probable in view of the subsequent close association of nucleolus and chromatin in the late prophase stages of the mother cell.

The significance of the synapsis stage is a topic that has been frequently discussed; many students of the cell have considered it to be the time of pairing of the paternal and maternal elements. Overton, however, says that in the plants with which he worked the prochromosomes are already paired at the stages of greatest chromatic distribution of the mother cell, and that the pairing may possibly occur in the telophases of the last pre-meiotic division. In these cases the individuals of a pair are very closely associated, in fact, so much so that in his earlier work the author considered each pair a single body. In *Acer platanoides* there may be a general grouping of the chromatin bodies into pairs even in the resting stages of the nucleus, but their close association does not take place until synapsis. This evidence is in accord with the belief that synapsis is a significant stage in the heterotypic division.

The manner in which the members of a pair of the chromatin bodies become joined together is shown to be an end-to-end arrangement; this fact is verified by the stages immediately following their union when the chromatin threads are formed by the flowing out of the chromatin in opposite directions from the paired chromatin bodies. There certainly is no evidence that I can find to support Cardiff's more or less diagrammatic figures of a side-by-side pairing before synapsis; his figures 5 to 13, inclusive, which he interprets as pre-synaptic, are more suggestive of post-synaptic stages; he calls attention to the fact that the threads are arranged in pairs and that most of the pairs seem to be in contact with the nucleolus, or very near it; this is a condition which I find in post-synapsis.

The interpretation of the nature of the pre-synaptic spirem has been the principal factor in the divergence of conclusions reached by the parasynaptic and telosynaptic schools. In forms like *Acer platanoides* and *Acer negundo*, as reported by Mottier, in which a pre-synaptic spirem does not occur, and in which the chromatin bodies are not split, this factor of whether the spirem is split or double is removed and the evidence for the manner of pairing becomes more conclusive.

The chromatic spirem in *Acer platanoides* is formed during the synaptic contraction by the flowing out of the chromatin-staining material along the linin in much the same manner as found by Overton in Thalictrum. Mottier apparently did not determine the behavior of the chromatin masses in *Acer negundo* during the synaptic stages, but he states that the spirem is formed during this stage.

The formation of the bivalent chromosomes from the thick chromatin

threads suggests nothing unusual, unless it be that some of the threads fold on themselves and some do not, as the examination of several cells has led me to suspect.

The direct origin of the chromosomes in the homoeotypic division from the chromatin masses in the daughter nuclei and the subsequent behavior of these chromosomes in the formation of the granddaughter nuclei are strong evidences in support of the theory of the permanence of the individual chromosomes.

Summary

1. Chromatin masses corresponding in number to the chromosomes at the time of cell division are present in the various stages of the vegetative cells and of the reproductive cells in *Acer platanoides*.

2. These chromatin masses may be followed through synapsis, in which stage they become closely paired and unite end to end.

3. The chromatin threads are formed by a flowing out of the chromatin material from the chromatin masses.

4. The chromosomes do not lose their individuality in passing through the telophase stages to the resting stage of the nucleus.

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EXPLANATION OF PLATES

All figures were made with the aid of a camera lucida with Spencer 1.5-mm. objective and 10x eyepiece. Drawings magnified about 2900 diameters.

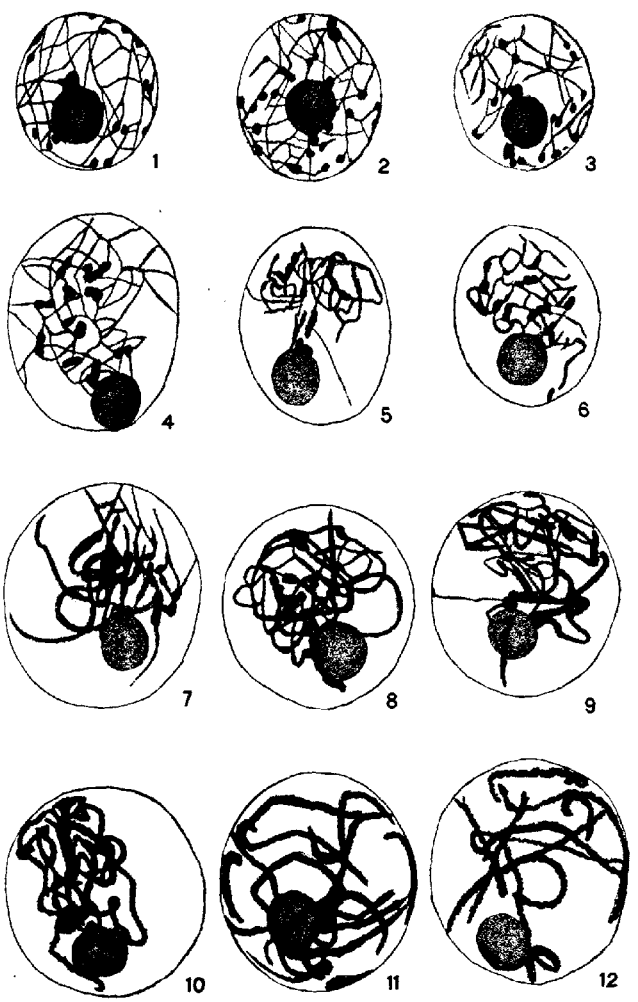
Plate XXXI

Fig. 1. Resting nucleus of the pollen mother cell, showing chromatin bodies, net-like linin, and bud on nucleolus.

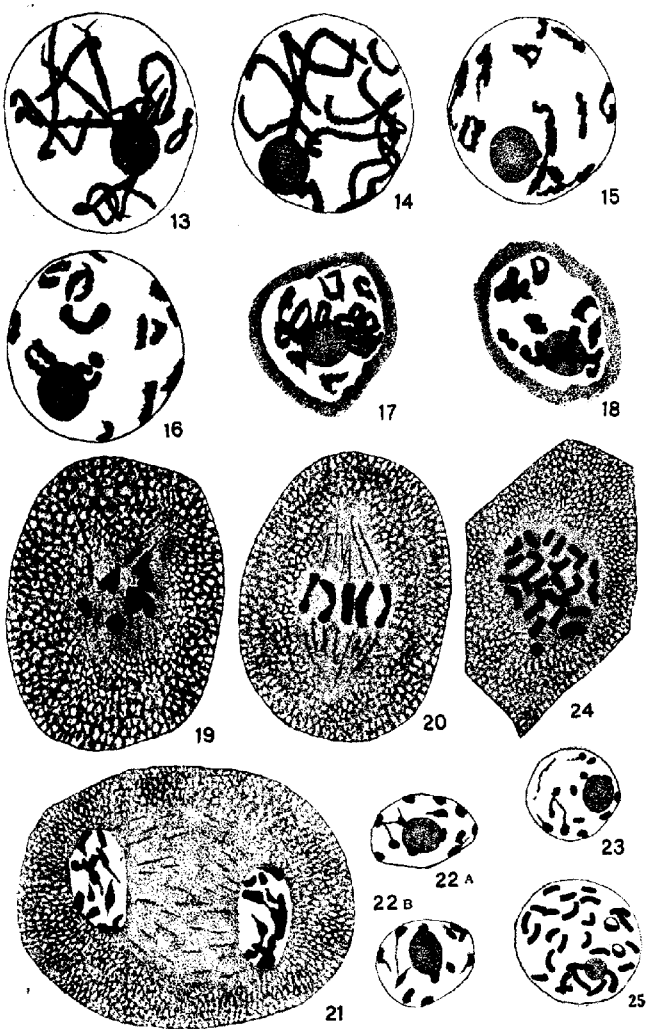
Fig. 2. Early growth period of the pollen-mother-cell nucleus, with a bud freed from the nucleolus.

Fig. 3. Early growth period of cell, showing conspicuous pairing of chromatin bodies.

Fig. 4. Early stage in synapsis, the linin becoming drawn into a mass and the chromatin bodies often joined together end to end in pairs.



DARLING: CHROMOSOME BEHAVIOR IN ACER



DARLING: CHROMOSOME BEHAVIOR IN ACER

FIGS. 5, 6. Portions of nuclei during early synapsis, the linin threads becoming thicker and the chromatin bodies more closely joined in pairs.

FIGS. 7, 8, 9. Stages in synapsis in which the chromatin becomes spread out from each pair of chromatin bodies to form the thick chromatin threads.

FIG. 10. Late synapsis, in which the chromatin bodies lose their identity in forming the chromatin threads.

FIG. 11. A stage in which the threads are becoming loosened from the synaptic knot.

FIG. 12. A portion of a nucleus in an early post-synapsis stage.

PLATE XXXII

FIG. 13. A post-synapsis stage, showing the split threads and the split granules on the threads.

FIG. 14. The chromatin threads, usually bent, are beginning to shorten.

FIGS. 15, 16. Diakinesis; some of the threads appear to be folded to form loops, some do not.

FIGS. 17, 18. The nuclear membrane disappearing and the chromosomes becoming more condensed and clustered about the nucleolus.

FIG. 19. A portion of a mother cell with spindle fibers becoming attached to chromosomes; some long chromosomes show the side-by-side pairing as a result of the folding of the chromatin thread.

FIG. 20. A portion of a mother cell in late metaphase; some bivalents are separated more than others.

FIG. 21. Early telophase; the chromosomes retaining their individuality.

FIGS. 22 *A*, 22 *B*. Resting daughter nuclei of a single cell, showing the thirteen chromosomes in each nucleus.

FIG. 23. A tetrad or granddaughter nucleus in the resting stage, showing the thirteen chromosomes.

FIG. 24. A polar view of the metaphase stage of a vegetative cell from the flower stalk, showing a general paired condition of the chromosomes and a discarded portion of the nucleolus.

FIG. 25. A late prophase stage of a vegetative cell from the flower stalk, showing a general pairing of the chromosomes.